Risk profile: *Vibrio parahaemolyticus* and *Vibrio vulnificus* in oysters and mussels in Tasmania

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This document was developed as part of project '2018-031: Assessing the Risk of Pathogenic *Vibrio* Species in Tasmanian Oysters' and was supported by funding from the Fisheries Research and Development Corporation on behalf of the Australian Government.

Information current as of 9th August 2023

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1. Introduction

1.1. Objective

To provide an initial qualitative risk assessment of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in Tasmanian aqua-cultured shellfish.

This risk profile will consider Pacific Oysters (*Magallana gigas*, previously *Crassostrea gigas*) and Blue Mussels (*Mytilus galloprovincialis*) in growing areas surveyed in the Fisheries Research and Development Corporation (FRDC) project 2018:031 "Assessing the risk of pathogenic *Vibrio* species in Tasmanian oysters". Shellfish imported from other Australian states or from overseas will not be considered in this risk profile.

1.2. Background

The halophilic bacterial genus, *Vibrio* is ubiquitous in marine and estuarine environments. Three species of the *Vibrio* genus, namely *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* are foodborne pathogens, the latter two being associated with seafood consumption, particularly bivalve molluscan shellfish (BMS) (Bonnin-Jusserand et al., 2019). Other occasional human pathogens in this genus are *Vibrio mimicus*, *Vibrio alginolyticus*, *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio metschnikovii* and *Vibrio hollisae* (Kaysner et al., 2004). BMS are considered a risk as they can accumulate and concentrate pathogenic bacteria from the marine environment through their filter feeding activity. Furthermore, often BMS are consumed raw increasing the risk of illness if these bacteria are present post-harvest (Ndraha et al., 2020). Illness from the consumption of *V. parahaemolyticus* and *V. vulnificus* contaminated seafood presents as gastroenteritis and occasionally as sepsis, with mortality occurring in immunocompromised patients (Bonnin-Jusserand et al., 2019). In the USA, foodborne vibriosis results in 52,000 cases annually (CDC, 2019). Until recently, there has been little data on shellfish borne vibriosis in Australia largely due to its historic sporadic nature, further hampered by varying jurisdictional requirement to report illness.

Vibrio have been detected in Australian oysters since the 1970s (Lewis et al., 2003). Most of these studies have been limited to point in time investigations using a variety of detection methodologies, hence making direct comparison of investigations challenging. Nevertheless, total and potentially pathogenic (*tdh*+ or *trh*+) *V. parahaemolyticus* have been detected in Sydney Rock Oysters (*Saccostrea glomerata*) and Pacific Oysters from New South Wales, Tasmania and South Australia since 2002 (Lewis et al., 2003, Madigan et al., 2007, Tamplin et al., 2011), and *V. vulnificus* has been detected in Pacific Oysters from Tasmania since 2016 (Madigan et al., 2017). Until recently there has been little epidemiological evidence supporting widespread non-sporadic foodborne illness associated with these pathogens from Australian shellfish consumption despite foodborne *V. parahaemolyticus* and *V. vulnificus* cases having been linked to Australian oyster consumption since the 1990s (Madigan et al., 2007, Harlock et al., 2022). The first Australian multi-jurisdictional outbreak of *V. parahaemolyticus* as a result of shellfish consumption was reported in January 2016 from Tasmania oysters grown in Moulting Bay with a total of eight illnesses confirmed (Madigan et al., 2017, Harlock et al., 2022). Two subsequent larger multidirectional outbreaks of *V. parahaemolyticus* linked to South Australian oysters were reported in 2021 (Government of South Australia, 2021, Leong et al., 2022).

The 2016, the Tasmanian vibriosis outbreak coincided with unusually warm seawater temperatures in the implicated growing area (Madigan et al., 2017). Furthermore, in 2015-2016 a marine heat wave was recorded in the Tasman Sea, with sea surface temperatures increasing 3-4 ℃ above average of south-eastern Australia (Oliver et al., 2017). The Tasman Sea has been identified as a hot spot for ocean

warming, with rapid increases of extreme heat wave events predicted to occur in the future (Oliver et al., 2015, Oliver and Holbrook, 2014). Marine heat waves have been associated with the emergence of *Vibrio* in other global temperate and cooler water shellfish growing areas, such as in northern Europe, the Baltic Sea, Chile and US Pacific northwest (Baker-Austin et al., 2013).

Following the 2016 Tasmanian vibriosis event, Madigan *et al*. (2017) undertook a limited *Vibrio* survey of oysters (n=30) from Moulting Bay between February and May of that year. Of the oysters sampled, 100% were found to contain *V. parahaemolyticus*, some with levels considered unsatisfactory from a food safety perspective, although temperature control post-harvest of samples could not be verified. Pathogenic *V. parahaemolyticus* (*tdh*+ or *trh*+) were also detected in approximately 20% of samples tested. Furthermore, 53% of oysters tested were also positive for *V. vulnificus* (Madigan et al., 2017).

Following this Tasmanian shellfish related vibriosis outbreak, a *Vibrio* Control Plan (VCP) was developed and implemented in Tasmania in 2018. The VCP is part of the "Food Safety Management System for Live Tasmanian Farmed Bivalve Molluscs" and implemented in growing areas from which vibriosis cases have been notified (Tasmanian Government, 2019a). Despite the implementation of the VCP, there have been on-going sporadic illnesses linked to Tasmanian oysters. One case was reported in 2017 related to recreational harvest of oysters and six cases in 2019 from commercially harvested oysters from various harvesting regions (Harlock et al., 2022). Since 2019, all zones of Big Bay, Moulting Bay, Great Swanport and Pipeclay Lagoon were obliged to follow the VCP. For all other growing areas, the VCP is recommended but not obligatory. Furthermore, in 2019 Oysters Tasmania published a *V. parahaemolyticus* guide for Tasmanian shellfish growers (Oysters Tasmania, 2019).

In 2019, a survey for total and pathogenic *V. parahaemolyticus* and *V. vulnificus* in Tasmanian shellfish commenced as part of a Fisheries Research and Development Corporation project: "FRDC 2018-031; Assessing the risk of pathogenic *Vibrio* species in Tasmanian oysters". The prevalence and levels of *Vibrio* was analysed from BMS (Pacific Oysters and Blue Mussels) from eleven Tasmanian harvest areas. Shellfish were sampled over three summer/autumn and two winter/spring periods between 2020-2022. In addition to the microbiological data, environmental data (growing water and ambient temperatures, salinity, rainfall, and river flow where applicable) were also analysed to determine the relationship to *Vibrio* risk in each area. The results from this survey have been used to develop this risk profile, the first such analysis undertaken for pre-harvest commercial shellfish in Australia.

2. Hazard

2.1. *Vibrio parahaemolyticus*

Vibrio parahaemolyticus is a halophilic Gram-negative rod shaped or singularly curved bacterium with a polar flagellum and numerous lateral flagella (Lu et al., 2019), frequently found in fresh seafood . It naturally occurs in brackish and coastal waters as a planktonic organism or attached to sediments, zooplankton, fish and shellfish (de Souza Santos et al., 2015). *Vibrio parahaemolyticus* was first isolated in Japan in 1950s by Tsunesaburo Fujino from "Shirasuboshi", a food preparation of semi dried sardine, where it caused 272 illnesses and 20 deaths during a large outbreak (Zen-Yoji et al., 1965, Fujino, 1953, Barker and Gangarosa, 1974). *Vibrio parahaemolyticus* is the most prevalent bacteria globally responsible for gastroenteritis, associated with seafood consumption (Baker-Austin et al., 2017, de Souza Santos et al., 2015). It can grow in a range of temperatures (5 – 43 °C), pH (4.8 – 11), and salt concentrations (0.5 – 10% NaCl). However, the optimal temperature, pH and salt requirements are 30 - 35 °C, pH 7.8 - 8.6, and 1% - 3% salt (Jones, 2014). They can grow either aerobically or anaerobically, but prefer oxygen for growth (Dabanch et al., 2009).

2.1.1. Pathogenicity and virulence

Pathogenicity and virulence can be defined in multiple ways (Thomas and Elkinton, 2004). Generally, pathogenicity is defined as the potential ability of a microorganism to cause disease in its host, while virulence is defined as the degree of pathogenicity as determined by the factors that make a microorganism pathogenic. Virulence factors are related to attachment, proliferation, immuno-evasion, immuno-suppression, destructive enzymes, lipopolysaccharides, iron uptake systems, outer membrane proteins and toxins encoded by pathogenic strains (Broberg et al., 2011, Li et al., 2019).

Not all strains of *V. parahaemolyticus* are pathogenic to humans. In environmental and food samples, only 0.3 to 3% of the total *V. parahaemolyticus* population induce clinical symptoms in humans (Cook et al., 2002, Kaufman et al., 2003). Pathogenicity of *V. parahaemolyticus* is principally believed to be related to the expression of two hemolysin proteins; the thermostable direct hemolysin (TDH), encoded by the *tdh* gene, and the TDH related hemolysin, encoded by the *trh* gene (Honda and Iida, 1993, Honda et al., 1988). Expression of the *tdh* gene is regulated by the *ToxR* gene, another potential virulence marker (Lin et al., 1993). Clinical strains of *V. parahaemolyticus* are largely hemolytic on Wagatsuma agar, termed the “Kanagwa phenomenon” (KP), and these strains carry the *tdh* gene (Miyamoto et al., 1969, Bhoopong et al., 2007). It was later observed that some clinical strains were KP negative but carried the *trh* gene for which there is currently no *in-vitro* test (Bhoopong et al., 2007, Ramamurthy and Nair, 2014, Kaysner et al., 2004). Both these hemolysins cause perforation in the gut leading to loss of ions and small molecules, and an uncontrollable water influx leading to diarrhea (Yanagihara et al., 2010). Urease activity encoded by *ure* gene in pathogenic *V. parahaemolyticus* strains was found to be linked with *trh*, suggesting that *ure* is another potential virulence marker (Caburlotto et al., 2009). Although the *tdh* and *trh* hemolysin genes have been considered to be primary virulence factors, there is evidence showing that they are not definitive virulence markers; these genes are frequently identified in non-clinical strains and at least 10% of clinical strains lack these genes (Jones et al., 2012b, Martinez-Urtaza et al., 2013). A range of other virulence factors have also been identified in *V. parahaemolyticus* responsible for clinical symptoms in humans or pathogenicity in animal models (**Table 1**).

Genes related to the type-III secretion systems (T3SS1 and T3SS2) and type-VI secretion systems (T6SS1 and T6SS2) have been identified to be related to *V. parahaemolyticus* virulence (**Table 1**) (Makino et al., 2003, Qiu et al., 2020). Bacterial secretion systems are dedicated to secreting a range of proteins required for their growth and survival. These can be exploited by some bacteria as virulence factors to cause disease in their host (Green and Mecsas, 2016). Genes with in the T3SS encode a syringe like transmembrane device to deliver effector proteins into the cell’s cytoplasm. T3SS1 and T3SS2 are responsible for cytotoxicity and enterotoxicity, which are required to establish diarrhea in the host (Kodama et al., 2007, Kodama et al., 2008). The T3SS1, found in both environmental and clinical *V. parahaemolyticus* isolates, contain genes which regulate biofilm formation, bacterial motility, cytotoxicity and fitness in the environment (Li et al., 2019). Two distinct lineages of T3SS2, T3SS2α and T3SS2β, are correlated with the presence of *tdh* and *trh* respectively, and are found in the 80kb *V. parahaemolyticus* pathogenicity island 7 (VPaI-7) (Okada et al., 2009). Other components of the T3SS2 are hydrophobic proteins, VopB2 (*VPA1362*) and VopD2 (*VPA1361*), which form a pore structure across the host’s cell membrane and exert cytotoxicity and enterotoxicity (Kodama et al., 2008). The T3SS2 also encodes a range of effector proteins, VopA/P, VopC, VopT, VopL, and VopV, which work together to promote bacterial colonisation of the host’s intestine and cause fluid accumulation and inflammation in the intestinal tract (Broberg et al., 2010, Kodama et al., 2007, Liverman et al., 2007).

The T6SS is a bacteriophage like protein injection system involved in symbiosis, biofilm formation, virulence, anti-pathogenesis, stress response and ion uptake (Silverman et al., 2012, Li et al., 2019). The T6SS1 of *V. parahaemolyticus* encodes an antibacterial system and adhesion proteins under warm marine conditions (30℃ and 3% NaCl), whereas the T6SS2 encode bacteria adhesion proteins and are activated by low salt concentrations (Yu et al., 2012, Li et al., 2019).

Other studies suggest *V. parahaemolyticus* pathogenicity may be related to other genes, or yet unknown genes which are not related to the known virulence factors (Wagley et al., 2018, Ceccarelli et al., 2013, Bhoopong et al., 2007). For instance, an adhesion protein, VpadF, has been identified in *V. parahaemolyticus* strains associated with lethality in a mouse model (Liu and Chen, 2015). MAM7 is another adhesion protein which is believed to be associated with pathogenicity of *V. parahaemolyticus* (Krachler et al., 2011). The *mutT* gene, which encoding a nudix hydrolase in some gram-negative bacteria, has been identified as a potential virulence marker using insect models. Clinical strains of *V. parahaemolyticus* containing *mutT*, but lacking *tdh*, *trh* and T3SS genes, have been shown to cause lethality in insect models (Wagley et al., 2018).

Despite the great body of work investigating clinical and environmental isolates, no definitive and universal virulence factors have yet been identified for *V. parahaemolyticus*.

Table 1: Some virulence factors for pathogenicity determination in *V. parahaemolyticus* (Zhang and Orth, 2013). Note this table is not comprehensive.

Genes	Domain	Activity	Function
Toxins and adhesin			
tdh	Thermostable direct hemolysin	Pore forming toxin	Causes cytotoxicity and enterotoxicity
trh	TDH related hemolysin	Pore forming toxin	Causes cytotoxicity and enterotoxicity
MAM7	mce domain	Binds to fibronectin and phospholipid phosphatidic acid	Forms attachment for the bacterium to a host cell
T3SS1 effectors			
***vopQ* (VP1680)**	Non-conserved	Binds to V-ATPase	Induces autophagy
***vopS* (VP1686)**	Fic domain	AMPylates Rho family GTPases	Disrupts actin cytoskeleton
Vpa0450	Inositol polyphosphate 5-phosphatase	Hydrolyses PI(4,5)P2 to PI4P	Disrupts plasma membrane integrity
T3SS2 effectors			
***vopC* (VPA1321)**	Cytotoxic necrotizing factor	Deamidates Rac and CDC42 at their switch-2 region	Disturbs actin network and causes bacterial invasion
v*opT* (VPA1327)	ADP-ribosyltransferase	ADP-ribosylates Ras	Unknown
***vopA/P* (VPA1346)**	Acetyltransferase	Inhibits MAPK signalling	Suppresses immune response
***vopV* (VPA1357)**	Non-conserved	Actin binding and bundling	Causes cytotoxicity and enterotoxicity
***vopL* (VPA1370)**	WH2 domains	Actin nucleation	Induction of actin stress fibre

2.1.2. Methods of detection

Several diagnostic methods, international standards and guidelines are available for the detection and quantification of *V. parahaemolyticus* in food and water (FAO/WHO, 2016, ISO, 2017, ISO, 2020, Kaysner et al., 2004, NSSP, 2019). The selection of diagnostic method, and consideration as to whether it should be qualitative (presence/absence) or quantitative (enumeration), is dependent on the intended application of results i.e. harvest area monitoring, post-harvest process verification, end product monitoring, or outbreak investigation (FAO/WHO, 2016). It is recommended that quantitative methods be used to evaluate concentrations of total and pathogenic *V. parahaemolyticus* strains in food implicated in outbreaks (FAO/WHO, 2016).

Diagnostic methods for *Vibrio* spp. in foods are generally based on a combination of initial microbiological enrichment and culture followed by biochemical or molecular characterisation (polymerase chain reaction; PCR) techniques, colony hybridisation with labelled probes or loop-mediated isothermal amplification; LAMP) (Jones et al., 2012a, ISO, 2017, ISO, 2020, Kaysner et al., 2004, FAO/WHO, 2016, NSSP, 2019). Common diagnostic methods include (FAO/WHO, 2016):

1. Enumeration by direct plating on selective media;
2. Direct plating on non-selective media followed by colony hybridisation with probes;
3. Conventional selective enrichment followed by:
    a. Selective plating and biochemical characterisation, or
    b. Molecular testing of presumptive isolates, or
    c. Direct molecular testing on the enrichment broth;
4. The most probable number (MPN) method involving enrichment in broth followed by:
    a. Plating and biochemical testing, or
    b. Plating and molecular testing, or
    c. Direct PCR on broth.

Direct plating on selective or non-selective media, or enrichment of seafood homogenate in alkaline peptone water (APW) prior to further characterisation are widely practiced across the various methods (FAO/WHO, 2016). The most popular selective media used are thiosulfate citrate bile-salts sucrose (TCBS) agar and *Vibrio* chromogenic agars. Presumptive positive *V. parahaemolyticus* colonies need to be confirmed by either biochemical or molecular techniques. Methods have also been developed to confirm *V. parahaemolyticus* directly by PCR from enriched samples prepared in a MPN format without the need for isolating colonies on selective or non-selective media (Nordstrom et al., 2007, Kinsey et al., 2015). PCR based detection techniques have become more popular than biochemical characterisation due to the greater accuracy in *V. parahaemolyticus* identification (Hartnell et al., 2019, ISO, 2017). Furthermore, reliable detection of enteropathogenic *V. parahaemolyticus*, as determined by presence of the *tdh* and *trh* genes, can only be done using molecular methods. Various gene regions and targets are recommended for the detection *V. parahaemolyticus* including the membrane-associated transcriptional factor gene (*ToxR*) and thermolabile hemolysis gene (*tlh*) for total *V. parahaemolyticus* and *tdh* and *trh* genes for pathogenic *V. parahaemolyticus* (ISO, 2017, Kaysner et al., 2004, NSSP, 2019, ISO, 2020). Target regions other than those specified in the ISO 21872-1 standard may be used if they demonstrate equivalent performance, are published in a peer reviewed journal and are verified against a broad range of target *Vibrio* spp. and non-target strains (ISO, 2017). Gene targets and testing applications for those included in the ISO 21872-1 have been validated in an international proficiency trial (Hartnell et al., 2019).

Non-selective enrichment is recommended for food/shellfish testing to elevate *Vibrio* numbers to detectable levels and to dilute potentially inhibitory effects of the food matrix (FAO/WHO, 2016). In general, direct detection, whether microbiological or DNA based, are less successful as the target *Vibrio* spp. may be present below the limit of sensitivity for the test, selective agars may be more inhibitory to target bacteria, and the shellfish matrix may inhibit molecular detection or growth of target species (FAO/WHO, 2016). Recovery of *Vibrio* spp. from foodstuffs may be further improved by altering incubation temperatures during enrichment, depending on the target species and state of food matrix. For example recovery of *V. parahaemolyticus* and *V. cholerae* in fresh seafood is enhanced by enrichment at 41.5°C, whereas in deep frozen (less than −18 °C), dried or salted products recovery of *V. parahaemolyticus, V. vulnificus* and *V. cholera* is enhanced by enrichment at 37°C (ISO, 2017). *Vibrio* can also enter a dormant or viable but non-culturable (VBNC) state during unfavourable environmental conditions (i.e., low nutrient concentrations, suboptimal and downshift temperatures, and elevated salinity) (Fernandez-Delgado et al., 2015). The use of post-harvest cold storage of shellfish may induce bacterial stress and a shift to the VBNC state, hence they are unable to grow on selective agar plates

and require a resuscitation step to improve recovery (FAO/WHO, 2016). Thus, the VBNC state can negatively impact the diagnostic outcome.

Qualitative diagnostic methods for *V. parahaemolyticus* are described in ISO 21872-1:2017 "Microbiology of the food chain — Horizontal method for the determination of *Vibrio* spp. — Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*" and the US FDA "Bacteriological Analytical Manual (BAM) Chapter 9: *Vibrio*" (Kaysner et al., 2004). Three quantitative methods for enumerating *V. parahaemolyticus* are described in the US FDA BAM, including an MPN method, a membrane filtration procedure using hydrophobic grid membrane filter (HGMF) and direct plating method using DNA probes for identification of the total and pathogenic *V. parahaemolyticus* (Kaysner et al., 2004). The ISO/TS 21872-2 "Microbiology of the food chain — Horizontal method for the determination of *Vibrio* spp. — Part 2: Enumeration of total and potentially enteropathogenic *V. parahaemolyticus* in seafood using nucleic acid hybridization" also described a direct plating enumeration method using DNA probes for total and pathogenic *V. parahaemolyticus* similar to the BAM (ISO, 2020). Although the membrane hybridisation method does provide enumeration it is laborious and less convenient than PCR based methods. The US National Shellfish Sanitation Program (NSSP) describes two validated MPN PCR based methods for enumerating either total *V. parahaemolyticus* or pathogenic *V. parahaemolyticus* (ISSC, 2015a, ISSC, 2015b). These are the methods on which we have based our testing during the Tasmanian *Vibrio* prevalence survey.

A quantitative Australian standard AS 5013.18 – 2010 "Food microbiology method 18: Examination for specific organisms – *Vibrio parahaemolyticus*" based on MPN and biochemical confirmation for *V. parahaemolyticus* was available until August 2022, when it was withdrawn (Australian Standard, 2010). No specific method for *Vibrio* testing is stated in the Australian Shellfish Quality Assurance Program (ASQAP) operations manual, other than laboratories undertaking analysis of water or shellfish need to be accredited to ISO/IEC 17025 from the National Association of Testing Authorities (NATA) or an equivalent body (ASQAAC, 2022). A list of Australian accredited commercial organisations for *Vibrio* testing are listed on the NATA website (NATA, 2023) Those laboratories which have been utilising AS 5013.18 – 2010 for quantification of *V. parahaemolyticus* will either need to validate the method or adopt another validated method. Currently only a few commercial laboratories offer validated quantitative methods for *V. parahaemolyticus*. A new qualitative (presence/absence) Australian standard (AS 5013.18.1) for *Vibrio* spp. was released in April 2023 which includes identification of *V. parahaemolyticus* and is a modification of the ISO 21872-1:2017 method (Australian Standard, 2023). The standard is based on primary and secondary enrichment followed by primary and secondary isolation and confirmation based on either biochemical testing, end-point PCR or real-time PCR, including provision for confirmation of *tdh*+ and *trh*+ *V. parahaemolyticus* strains by PCR*,* which was not available in the previous AS 5013.18 standard.

Enumeration of *Vibrio* can be reported as either colony forming units (CFU) per gram of food or most probable number (MPN) per gram of food. CFU/g are reported when using direct plating methods which enumerate the viable bacterial cells in each sample on solid media and generates a continuous result (FAO/WHO, 2016). MPN/g estimates viable cells in a liquid sample using 3, 5 or 10-tube multiple dilution, and is a statistical method based on probability with 95% confident limit. Hence, this method is considered semi-quantitative. The limit of detection of MPN/g tends to be lower than CFU/g and thus MPN in conjunction with qPCR is particularly useful when analysing samples which contain low concentrations of bacterial cells (FAO/WHO, 2016).

V. parahaemolyticus strains have traditionally been classified using their antigenic properties (somatic; O or capsular; K antigens), although DNA fingerprinting techniques, such a s multi-locus sequence typing (MLST) and whole genome sequencing (WGS) are becoming more popular and available in

epidemiologic investigations (Kaysner et al., 2004, González-Escalona et al., 2008, Han et al., 2016, Jesser et al., 2019).

2.1.3. Environmental strains

Vibrio parahaemolyticus naturally occurs in marine waters. They have significant correlation with warm (>15 ℃) and low salinity (<25 ppt NaCl) water (Baker-Austin et al., 2010, Oberbeckmann et al., 2012). They are often associated with chitinous organisms such as zooplanktons, algae and other particulates and may survive within free living protozoa (Thomas et al., 2010). *Vibrio* are spread globally by importation of live bivalves into local waters (Martinez-Urtaza et al., 2013), ballast water movements (Depaola et al., 1992), migratory birds (Fu et al., 2019) and through long distance oceanic transport into a pristine region (Martinez-Urtaza et al., 2008). *Vibrio parahaemolyticus* plays important roles in nitrogen cycling, chitin degradation and hydrocarbon degradation in the environment (Greenfield et al., 2017, Criminger et al., 2007). The hemolysin genes, *tdh* and *trh*, often associated with virulence, are generally absent in environmental strains of *V. parahaemolyticus*. However their presence in some environmental strains suggests a role of these genes in fitness of the organism to its environmental ecosystem (Li et al., 2019).

During winter, *V. parahaemolyticus* is generally not detected in the water or shellfish but remains detectable in sediments (Kaneko and Colwell, 1975) and is reintroduced into the water column as temperature rises (Su and Liu, 2007). In the warmer seasons, they can be detected in high numbers (ca. 150 – 46,000 *V. parahaemolyticus* per gram) in shellfish (Urquhart et al., 2016, Parveen et al., 2008, DePaola et al., 2000); sometimes 100 times more than in the surrounding water (DePaola et al., 1990, Broberg et al., 2011). Global warming and low salinity of sea water trigger the emergence of pathogenic strains of *V. parahaemolyticus*, especially in temperate regions (Baker-Austin et al., 2013). Modelling has suggested sea surface temperatures have risen up to 1.5ºC in the last 54 years and this is potentially linked with increasing cases of vibriosis in northern Europe and the Atlantic coast of the USA (Vezzulli et al., 2016). However, in regions where water temperatures are stable, other factors such as salinity, chlorophyll a, phytoplankton, copepods, turbidity and nutrients are known to drive the dynamics of *Vibrio* communities (Wong et al., 2019, Padovan et al., 2021, López-Hernández et al., 2015, Julie et al., 2010). Some studies have reported a strong association of algal blooms (such as radiophytes, diatoms and dinoflagellates) with the emergence of *Vibrio* organisms in the marine environments (Greenfield et al., 2017, Julie et al., 2010). Environmental communities of *V. parahaemolyticus* may also demonstrate strong positive correlation with factors that drive algal blooms, such as with dissolved organic matter, silicate, dissolved phosphate, dissolved oxygen, dissolved nitrogen and organic nitrogen in marine waters (Turner et al., 2014, López-Hernández et al., 2015). In contrast, a recent study in the northeast USA suggested there are no significant correlations between chlorophyll a, plankton and nutrients with the abundance of *V. parahaemolyticus* in the areas studied (Hartwick et al., 2021). Several *Vibrio* spp., including *V. parahaemolyticus* and *V. vulnificus*, can form biofilms on seafood and surfaces that come into contact with food (Ashrafudoulla et al., 2021). Biofilm formation along with antimicrobial drug resistance, hydrophobicity, quorum sensing and motility all contribute to the survival of *Vibrio* spp. in the environment (Ashrafudoulla et al., 2021).

2.1.4. Strains and their typing

Serotyping and genotyping are the two principal methods available for *V. parahaemolyticus* strain typing. Both are valuable epidemiological tools during foodborne investigations, although the two systems show little correlation. It should be noted that serological tests alone are not useful in identifying *V. parahaemolyticus*, due to the cross reactivity of the antisera with other organisms (Kaysner et al., 2004). Serotyping in *V. parahaemolyticus* involves characterisation of the bacterial somatic (O) and capsular (K) antigens with commercially available antisera. Currently, 13 O and 71 K antigens can be typed (Oliver and Jones, 2015). As multiple K antigens have been found to occur with some O antigens, this has resulted in numerous recognised O:K serotypes. This classification system was the first introduced in the

early 70s for epidemiological investigation (Fishbein and Wentz, 1973, Sakazaki, 1971). Although, some serotypes have been commonly found associated with various foodborne outbreaks, serotyping is unable to predict presence of pathogenic strains (Li et al., 2016). Moreover, serotyping cannot differentiate between sero-variants within a certain serotype. The establishment of genotyping has enabled better discrimination among strains. Methods used include PCR profiling of strains or DNA fingerprinting by Pulsed Field Gel Electrophoresis (PFGE), Random Amplified Polymorphic DNA (RAPD) and Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) (Martinez-Urtaza et al., 2016, Martinez-Urtaza et al., 2013, Meparambu Prabhakaran et al., 2020). Subsequently, Multi-Locus Sequence Typing (MLST), Whole Genome Sequencing (WGS) and Protein Motif Fingerprinting have all been used to determine phylogenetic relatedness of clinical and environmental strains (Jesser et al., 2019, Hou et al., 2006).

Multi-Locus Sequence Typing was first introduced in 2004 to characterise the pandemic O3:K6 strains which caused the 1996 vibriosis outbreak in India (Chowdhury et al., 2004). This study exploited four housekeeping genes (*recA*, *dnaE*, *gyrB* and *gnd*) and demonstrated that 94% of O3:K6 strains possessed an identical genetic profile (Chowdhury et al., 2004). This approach was further modified by González-Escalona et al. (2008) and is now widely used for pandemic strain characterisation. Currently MLST involves analysing seven housekeeping genes (*recA, dnaE, gyrB, dtdS, pntA, pyrC,* and *tnaA*) of *V. parahaemolyticus* by PCR and sanger sequencing, or *in-silico* analysis of WGS (González-Escalona et al., 2008). This method is well established, with a public database accessible online for interlaboratory data comparisons (https://pubmlst.org/organisms/vibrio-parahaemolyticus/). The MLST approach has been used to determine the evolutionary origin of the pandemic O3:K6 strain (Muñoz et al., 2014). Genetic analyses has revealed that serotypes O4:K68, O1:K25, and O1:KUT are genetically similar and to have evolved from the pandemic O3:K6 strain (Ramamurthy and Nair, 2014). Serologically identical strains are often reported to belong to different MLST sequence types (STs), for example some pandemic strains of O3:K6 have been identified as ST3 and ST1109, while some pandemic strains of O1:KUT have been identified as ST276, ST212, ST1002, ST1007 and ST1105 (Li et al., 2016, Yang et al., 2022). Pandemic strains of O3:K6 have demonstrated a high level of sequence diversity (e.g. ST3, ST227, ST431, ST435, ST487, ST489, ST526, and ST672) (Han et al., 2017).

With the advancement, accessibility and affordability of sequencing technologies, WGS has become more widely used, and the methodology of choice, for more accurate typing of *V. parahaemolyticus* strains due to its higher genomic resolution capability (Jesser et al., 2019, Whistler et al., 2015, Turner et al., 2016, Xu et al., 2017) as compared to MLST which only covers a small portion of the genome (~3 kb in MLST versus ~4 Mb in WGS) (Jesser et al., 2019).

2.1.5. Global pandemic strains

Since its isolation in Japan in the 1950s, *V. parahaemolyticus* has been identified as a major microbiological cause of seafood-borne illness globally, linked with the emergence of pandemic strains (Abanto et al., 2020). The global expansion of non-sporadic *V. parahaemolyticus* foodborne outbreaks began in the early 1990s (Abanto et al., 2020). A pandemic strain of *V. parahaemolyticus*, serotype O3:K6 (ST3), first emerged in India in 1996 and within a few months it was detected in Vietnam, Indonesia, Bangladesh, Laos, Japan, Korea, and Thailand, reaching the northwest region of the USA by 1998 and impacting almost all continents, including Asia, America, Africa and Europe (Abanto et al., 2020, Ceccarelli et al., 2013, Caro-Castro et al., 2020, Nair et al., 2007a). Several pandemic strains including O1:K25, O4:K68, O1:K41, O4:K12 were detected in Thailand between 1998 and 2002, of which, O1:K25 and O4:K68 are closely related and believed to have diverged from the O3:K6 serotype (Bhoopong et al., 2007). Outbreaks and illness commonly occur in the USA (CDC, 2019) and China (Yang et al., 2022).

In 2012 a new strain, ST36 (serotype O4:K12 and O4:KUT), was detected outside the Pacific northwest region of the USA where it originated (Turner et al., 2013). ST36 caused shellfish related gastroenteritis in multiple USA states and has become an ongoing issue in diverse geographical regions, including southern America and Europe (Martinez-Urtaza et al., 2013, Abanto et al., 2020). Another pandemic *V. parahaemolyticus* strain ST120 (serotype O3:K59) was detected in Peru in 2009. ST120 originated in China in the 1990s but later emerged in Latin American waters and was responsible for the 2009 summer foodborne outbreak (Gonzalez-Escalona et al., 2016).

Of particular interest are the pandemic strains that appear to have a cold-water tolerance. These strains can grow faster at cooler temperatures (Davis, 2008) and generally cause less severe clinical symptoms (Bag et al., 1999). Five pandemic *V. parahaemolyticus* strains isolated from India in the late 1990s and representing serotypes O3:K6, O1:KUK and O4:K68 (all ST1) were shown to grow almost two times faster than non-pandemic strains at 12℃ (Davis, 2008). Genetic analysis of environmental *V. parahaemolyticus* strains from New Hampshire, USA showed that colder water isolates (<11°C) were less diverse than those collected in warmer waters. (Ellis et al., 2012).

Pandemic strains of *V. parahaemolyticus* contain *tdh* gene but generally lack the *trh* gene (Han et al., 2017, Davis, 2008, Martinez-Urtaza et al., 2005). Various modes of global distribution of these pandemic strains have been suggested, ranging from importation of live shellfish, ballistic water movement and transport on oceanic currents (Abanto et al., 2020). Moreover, emergence of the sequence types ST3, ST36 and ST120 in Peru were linked with *El Niño*, a condition of heavy rainfall and heat waves (Martinez-Urtaza et al., 2008, Caro-Castro et al., 2020, Gonzalez-Escalona et al., 2016).
Vibrio parahaemolyticus strain types associated with clinical illness are rarely detected in shellfish or marine environments. Furthermore, a greater variety of strain types are detected in environmental samples as compared to clinical samples (Miller et al., 2021). WGS analysis of pandemic clinical ST3, ST36 and ST65 strains have suggested they contain extra gene functions as compared to other environmental ST types, enabling them to spend more time in the dormant state within the marine environment and hence are more difficult to detect. Furthermore, these isolates may also contain additional genes enabling them to take advantage of the nutrient rich intestinal environment to outcompete other environmental stains (Miller et al., 2021).

The pandemic ST36 *V. parahaemolyticus* strain has recently been identified in Australian and New Zealand commercial shellfish related outbreaks (Government of South Australia, 2021, Harrison, 2022). Between January and April 2021, 21 cases of foodborne vibriosis were reported in multiple jurisdictions across Australia, implicating commercial South Australian oysters. All clinical samples which underwent WGS featured a highly related ST36 (Government of South Australia, 2021). Later that same year 268 cases of foodborne vibriosis were reported from multiple jurisdictions, again implicating South Australian oysters (Government of South Australia, 2022). This subsequent outbreak was predominately related to *V. parahaemolyticus* ST417 (n=143) followed by ST50 (n=70) (Leong et al., 2022, Government of South Australia, 2022). ST417 was also isolated from oyster samples sourced from the same growing area. ST50 has also been identified in New Zealand seafood related vibriosis, although a greater variety of clinical ST types have recently been identified and associated with a variety of commercial and recreational seafood (FoodSafetyNews, 2022, Harrison, 2022). The ST417 has previously been identified in clinical specimens from Canada and south-eastern China (Banerjee et al., 2014, Chen et al., 2016).

2.2. *Vibrio vulnificus*

Vibrio vulnificus is a natural inhabitant of the marine ecosystem where shellfish grow and a significant concern for seafood food safety. *Vibrio vulnificus* is a halophilic gram negative curved-rod flagellated bacterium found in seafood, marine waters and sediments. The optimum temperature, pH and salinity for

its growth are 37°C (range 8 – 43°C), 7.8 (range 5 – 10) and 2.5% (range 0.5 – 5% NaCl), respectively (Ministry for Primary Industries NZ, 2001). It can grow under both aerobic and anaerobic conditions and is not associated with faecal contamination in the water (Bross et al., 2007, Ministry for Primary Industries NZ, 2001).

2.2.1. Pathogenicity and virulence

Available phenotypic and genotypic tools have not been able to clearly predict pathogenicity of *V. vulnificus* in humans (Baker-Austin et al., 2010, Hernández-Cabanyero and Amaro, 2020, López-Pérez et al., 2019). However, based on biochemical properties, serology, host range and phylogeny of the core genome, *V. vulnificus* has been subdivided into three biotypes (López-Pérez et al., 2019, Biosca et al., 1996).

Methods used to distinguish among biotypes include: the slide agglutination test with anti-biotype sera; dot blot assays; sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting for lipopolysaccharides (LPS) and outer membrane proteins (OMP); and sequencing of OMP encoding genes (Bisharat et al., 2007). Biotype 1 consists mostly of clinical strains, and are indole positive with several distinct LPS types (Biosca et al., 1996). Biotype 2 are predominantly pathogenic to aquatic vertebrates, but can cause opportunistic infection in humans, and are indole negative with a common LPS type (Biosca et al., 1996, Amaro and Biosca, 1996). Biotype 3 is geographically restricted to Israel where it caused an outbreak of wound infections among fish farmers and consumers of Tilapia and Carp in the mid-1990s (López-Pérez et al., 2019, Roig et al., 2018, Zaidenstein et al., 2008).

The 16S rRNA gene has also been used to classify *V. vulnificus* strains into types A, B and AB (Nilsson et al., 2003, Aznar et al., 1994, Kirs et al., 2011). Several studies have investigated the correlation of *V. vulnificus* 16S rRNA gene type with clinical or environmental origin, with contradictory results suggesting that the 16S rRNA types are not reliable virulence markers (Nilsson et al., 2003, Gordon et al., 2008, Kirs et al., 2011).

The presence of a virulence correlated gene (*vcg*) has been reported in *V. vulnificus* strains of biotype 1 (Warner and Oliver, 2008, Rosche et al., 2010). Polymorphisms within the *vcg* gene have been used to distinguish between clinical isolates (*vcg* type C) and environmental isolates (*vcg* type E) (Nilsson et al., 2003, Vickery et al., 2007). However, several subsequent studies have reported a large number of clinical isolates as *vcg* type E (Thiaville et al., 2011, Bier et al., 2013). Hence, the *vcg* gene is also not a reliable marker for pathogenicity. Phylogenetic studies have revealed that clinical strains have diverged from all other clades of *V. vulnificus* (López-Pérez et al., 2019). Studies have identified several potential virulence factors including capsular polysaccharide, siderophores, hemolysin, acid neutralizing pathways, attachment and adhesion proteins, protease, phospholipase and T6SS; although, these virulence factors are also often shared between clinical and environmental strains (Baker-Austin et al., 2017, López-Pérez et al., 2019, Wright and Morris, 2003).

Due to the severity of disease induced by *V. vulnificus*, along with the difficulty in discriminating between virulent and avirulent strains, all strains are treated as pathogenic. Pathogenicity is also thought to be related to the gender of host, host iron level and immunity status (Wright and Morris, 2003, López-Pérez et al., 2019).

2.2.2. Methods of detection

Several diagnostic methods, international standards and guidelines are available for the detection and quantification of *V. vulnificus* in food (FAO/WHO, 2016, ISO, 2017, Kaysner et al., 2004, NSSP, 2019). As for *V. parahaemolyticus* common considerations are selection of diagnostic method appropriate for intended application, incubation temperature, enrichment and media selection and the potential to enter a VBNC state (see section 2.1.2 for more detail).

For the detection of *V. vulnificus* in seafood, environmental, and clinical samples, enrichment in APW and isolation on colistin-polymyxin B cellobiose (CPC) agar, TCBS agar or Chromogenic *Vibrio* agar are all practiced (Jones, 2014, FAO/WHO, 2016). *Vibrio vulnificus* appears as yellow colonies on CPC-based media, however, other marine cellobiose-positive *Vibrio* spp. may also appear yellow. TCBS promotes the growth of other *Vibrio* spp. with similar colony morphologies including *V. parahaemolyticus*. Therefore, subsequent confirmation of the presumptive isolates is required. Common molecular methods for *V. vulnificus* confirmation include DNA colony hybridization, PCR, real-time PCR and LAMP. Molecular methods target the *vvhA* gene (FAO/WHO, 2016), which is a widely accepted genetic marker for the detection of *V. vulnificus* (Campbell and Wright, 2003).

Although, there are well recognised virulence markers for *V. parahaemolyticus*, these are lacking for *V. vulnificus*, with all strains treated as virulent.

The International Organization for Standardization (ISO) describes various qualitative methods for the detection of *V. vulnificus* (ISO, 2017). The US FDA BAM describes two analytical methods for enumerating *V. vulnificus* including MPN and the direct plating method using DNA hybridisation probes for identification (Kaysner et al., 2004). The NSSP describes three approved methods for enumeration of *V. vulnificus*; enzyme immunoassay, MPN followed by biochemical analysis or probe hybridisation or MPN followed by real-time PCR (NSSP, 2019, Kinsey et al., 2015). Until 2023 Australia did not have an analytical standard for *V. vulnificus* detection in food (Australian Standard, 2023). The new standard AS 5013.18.1. is a qualitative method which is a modification of ISO 21872-1:2017 and based on primary and secondary enrichment followed by primary and secondary isolation and confirmation based on either biochemical testing, end-point PCR or real-time PCR.

2.2.3. Environmental strains

In the environment, *V. vulnificus* are associated with plankton, sediments, shellfish and fish across a wide variety of salinities and temperatures. They are generally detected in warmer marine environments when the temperature is above 20°C (Cruz et al., 2016), however, they can thrive in a range of water temperature (7 – 36°C) (Motes et al., 1998, Deeb et al., 2018). In the warmer seasons, when bacterial numbers may be higher, filter feeding shellfish can concentrate bacteria, elevating numbers further. Some studies suggest salinity of 15 – 25 ppt (Cruz et al., 2016) or 5 – 20 ppt (Wetz et al., 2014) to be ideal for their growth in water, whereas higher salinity may hinder their propagation (Mccoubrey, 1996, Deeb et al., 2018). The organism is reported to decline in numbers in seawater with salinities of 30 ppt or more (Kaspar and Tamplin, 1993b) and relaying shellfish in water of “high” salinity (above 2.8%; 28 ppt) resulted in more rapid reduction of *V. vulnificus* numbers compared to holding them in waters of “moderate” salinity (13-18 ppt) (Parveen et al., 2017). Much of Australia’s seawater would be classified as “high” salinity by this criteria. Deeb et al. (2018) reported high growth of *V. vulnificus* in very low salinity of 2 – 3 ppt, which is out of the range of published optimum salinity. Other marine environmental factors, including chlorophyll a, phytoplankton, copepods, turbidity and nutrients, are reported to have an impact on the overall *Vibrio* community including *V. vulnificus* (Wong et al., 2019, Padovan et al., 2021, López-Hernández et al., 2015, Julie et al., 2010). Deeb et al. (2018) reported a positive correlation between turbidity and *V. vulnificus* and Padovan et al. (2021) reported positive correlation between total nitrogen, total phosphate, turbidity and *V. vulnificus* abundance in marine water.

3. Food: bivalve molluscan shellfish

3.1. Consumption

The major Australian aquaculture and commercial wild caught BMS species harvested are oysters, mussels, scallops and pipis (Steven et al., 2021). In Australia, oysters are generally eaten raw or lightly cooked, while mussels are generally eaten cooked but can also be eaten raw or pickled. Other bivalve shellfish species tend to be cooked, pickled or smoked. Bivalve shellfish eaten raw can pose a significant foodborne risk if contaminated with human pathogenic bacteria or viruses.

3.2. Biology

Bivalve molluscan shellfish are found in aquatic environments; the majority being marine species. They feed by passing water through their gills to filter out organic food particles, such as plankton and other detritus. They tend to be mostly immobile, settling in the one place as adults. Mussels can attach themselves to hard surfaces using strong silky fibres or 'byssus threads', clams such as pipis burrow into the sand, oysters can bond themselves to a rock surface while scallops can be more motile, sitting on the seafloor and moving from place to place (Australian Museum, 2022).

Bivalve molluscan shellfish are hermaphrodites and most are broadcast spawners, meaning that their eggs are fertilized outside their bodies (Creswell et al., 2018). Bivalves become conditioned to spawn at a time that will optimise the survival of the planktonic larvae.

Tasmania has four oyster hatcheries on the east coast producing both diploid and triploid Pacific Oyster spat (Personal communication Brown, 2022, Oysters Tasmania, 2022). Diploid oysters spawn while triploid oysters are selectively bred and sterile, growing faster as energy is not diverted to reproduction resulting in loss of meat condition. Spawning of Tasmanian diploid Pacific Oysters is triggered by increases in temperature of +5°C. Characteristically, spawning occurs during spring tide conditions, high flow and high temperatures, although each bay is different (Personal communication Huddlestone, 2022). Generally spawning occurs from late January through to March, followed by a further 1-2 months before diploid Pacific Oyster meat condition rejuvenates. During this time diploid oysters are not harvested and triploid Pacific Oyster are used to fill this harvest window; diploids tend to return to harvest condition in the lead up to Easter in Autumn (Personal communication Huddlestone, 2022).

3.3. Commercial production – aquaculture and wild catch

In 2019-20, the national aquaculture production of oysters was valued at $114.4 million (9,011 tonne) and $6 million (2,342 tonne) for Blue Mussels (*Mytilus galloprovincialis*) (Steven et al., 2021). For the same period, Tasmanian aquaculture oysters were valued at $30.7 million (2,883 tonne) and Blue Mussels were valued at $2.3 million (572 tonne), representing 29% and 38% of the national value of production for oyster and mussels, respectively (Steven et al., 2021). In 2019-20, the national production of commercial wild caught scallops was valued at $18 million (6,615 tonne); the Australian Bureau of Statistics (ABS) has not reported commercial Tasmanian wild caught scallop harvest since 2016-17 (Steven et al., 2021). In 2019-20, national commercial wild caught pipis were valued at $7.8 million (603 tonne), with 88% of production coming from New South Wales and South Australia (Steven et al., 2021). Between 2018-2020 the export of Australian BMS was minimal, and largely consisted of scallops at 256 to 285 tonnes per year (with only Queensland and Western Australia reporting scallop production during this period) (Steven et al., 2021). Although Tasmanian Pacific Oyster export volumes are not reported in the ABS due to the low volume of exports, they are exported to Singapore and Japan (Personal communication Huddlestone, 2022). Imports of BMS during this period were largely from mussels and scallops at 2,959 to 3,243 tonnes and 1,738 to 1,917 tonnes per year, respectively (Steven et al., 2021).

Oyster production in Australia is dominated by the cultivation of two species, namely Sydney Rock Oysters (*Saccostrea glomerata*) and Pacific Oysters (*Magallana gigas*). Smaller numbers of Native Oysters (*Ostrea angasi*) and the tropical Blacklip Rock Oysters (*Saccostrea echinata*) are also produced in some locations (Myers and Stephens, 2020). Ninety-nine percent of Australia's oyster production occurs in New South Wales, South Australia and Tasmania, with cultivation focusing on cooler water species such as Sydney Rock Oysters and Pacific Oysters (Myers and Stephens, 2020).

The Tasmanian oyster industry is focussed on the aquaculture production of Pacific Oysters (Myers and Stephens, 2020). Spat are obtained from commercial hatcheries that breed for select families that have desirable traits e.g., fast growth, condition, shape and disease resistance, and are grown out in various embayment's throughout the state (Myers and Stephens, 2020). Pacific Oysters are the most common oyster species cultivated globally. They grow very rapidly and can reach a marketable size within 12 months in some regions. They are not a native species to Australia, having been introduced into Tasmania in the 1940's for aquaculture purposes (Myers and Stephens, 2020). Adult Pacific Oysters are sessile and can be found naturally on a variety of hard substrates in the intertidal and shallow subtidal zones, to depths of approximately 3 metres; they favour brackish, cool waters in sheltered waterways, but can tolerate a wide range of salinities and can occur offshore (Myers and Stephens, 2020).

Pacific Oysters are grown around the north-west, east and south-east coasts of Tasmania from the far north-west coast through to the southern part of the D'Entrecasteaux Channel, south of Hobart. Most areas used by the oyster industry are leased within the intertidal zone, although there are some deeper water areas. There are over 100 license holders and approximately 1356 ha in cultivation (Oysters Tasmania, 2022). Approximately 80% of annual production comes Pipe Clay Lagoon, Pitt Water, Moulting Bay and Boomer Bay, while approximately 8% of production comes from the Far North West, (King Island and Port Sorel) and 7% from the D'Entrecasteaux Channel and Southern Tasmania (Personal communication Brown, 2022). The majority of oyster harvest occurs between July and December (Grau, 2020). Harvest volumes from the Far North tend to be lower between May to October due to rainfall closures. The peak harvest times are during the festive seasons of Christmas and Easter, with a third of all sales occurring between Christmas and the New Year (Personal communication Huddlestone, 2022). Approximately 85% of Tasmanian oysters are sold within Australia, outside of Tasmania (Grau, 2020).

Tasmania also has a small aquaculture production of Native Oysters, often referred to as angasi, flat, mud or Port Lincoln oysters. Native Oysters are endemic to southern Australia, with a wide distribution from Western Australia to New South Wales and around Tasmania, where they inhabit intertidal regions to depths of 30 m (Crawford, 2017). Historically, this species has been an important component of the Australian Aboriginal diet. However, its low-profile reef bed habitat has become diminished due to destructive harvesting following European colonisation. Currently the only known remaining commercial Native Oyster reef habitat in its entire distribution is a few hectares in Moulting Bay (Crawford, 2017). Only a few oyster growers are producing commercially viable quantities of Native Oysters as they are more difficult to grow than major commercial oyster species. Native Oysters are naturally more abundant in the subtidal zone but can occur in the intertidal zone. Compared to other oyster species they are less tolerant of fluctuating environmental conditions, especially temperature and salinity, preferring cooler water and normally growing lower in the water column than Pacific and Sydney Rock Oysters (Crawford, 2017). They also have a shorter shelf life, gaping one to two days after harvest (Crawford, 2017). However, Native Oysters kept moist, prechilled to < 3°C and tightly packed in polystyrene boxes and stored at 4.5°C can remain fresh for 14 days. It is recommended that Native Oysters be chilled to 0.5 - 2°C immediately after harvest to minimise the growth of bacteria (Crawford, 2017). Guidance on post-harvest temperature controls for Pacific and Native Oysters for human consumption are similar and more stringent than for Sydney Rock Oysters (NSW Food Authority, 2018, ASQAAC, 2022). A commercial

dive fishery for Native Oysters has operated in Moulting Bay since 1985 and the fishery operates on mixed species shellfish beds. The fishing year operates from 1st September to 31st August (Keane and Gardner, 2018). The total allowable catch for commercial wild-caught Native Oysters in Tasmania in the 2021/22 season was 32.2 tonne (Tasmanian Government, 2021b). Native Oysters' reproduction differs to that of other commercial oyster species in that they are not broadcast spawners. Instead, eggs are fertilised within the female and the developing larvae maintained within the mantle before breaking free and eventually resting on sand and soft mud sediments. Native Oysters are slower growing than Pacific Oysters. Pacific Oysters typically take 1-2 years to reach harvestable size whereas Native Oysters take 2-4 years (Crawford, 2017).

Tasmania also has numerous other commercial wild catch BMS fisheries, which include Venus Clam (*Venerupis largillierti*), Native Oysters, Vongole Cockles (*Katelysia scalarina*), Commercial Scallops (*Pecten fumatus*) and wild Pacific Oysters (Tasmanian Government, 2021b, Keane and Gardner, 2018). A commercial dive fishery for the Venus Clam has operated in Moulting Bay, Tasmania since approximately 1985 (Keane and Gardner, 2018). The Venus Clam is endemic to New Zealand but was found in Tasmania in 1963. Venus Clams are found in the intertidal zone and subtidally, in both muddy and sandy substrates in shallow estuarine waters on parts of Tasmania's east and south-east coasts. The total allowable commercial catch for Venus Clams in Tasmania in the 2021/22 season was 4.4 tonnes (Tasmanian Government, 2021b). The total allowable catch for wild bivalve shellfish species is determined by stock surveys undertaken every two to three years. Currently, there is no cap on the number of wild Pacific Oyster licences that can be issued, but as for all commercial bivalve fisheries (aquaculture and wild-caught), operators are bound by the requirements of the Tasmanian Shellfish Market Access Program (ShellMAP) Regulatory Services and liable to pay the associated levy (Tasmanian Government, 2021b).

A commercial fishery for Vongole Cockles exists at Ansons Bay, northeast Tasmania, but was classified as environmentally limited in 2015 and has since remained closed to commercial harvest (Keane and Gardner, 2018). Vongole Cockles are found state-wide in Tasmania, but the commercial fishery has been restricted to Ansons Bay. Vongole Cockles are an estuarine intertidal species adapted to the large-scale salinity fluxes typical of Tasmania's east coast estuaries to which they respond like many other shallow-water bivalves by closing their valves (Tarbath and Gardner, 2015). The habitat of Vongole Cockles is intertidal, soft sediments in sheltered habitats at a depth of 2-4 cm. Spawning is predominantly in spring and summer (Tarbath and Gardner, 2015).

Commercial wild-caught scallop fishing began in Tasmania in the early 20th century, targeting the Commercial Scallop, one of three species naturally occurring in Tasmania: the others being Doughboy (*Mimachlamys asperrima*) and Queen Scallop (*Equichlamys bifrons*) (Semmens et al., 2020). The Tasmanian Scallop Fishery extends to 200 nautical miles from the Tasmanian coast, apart from Bass Strait, where its jurisdiction covers 3-20 nautical miles (Tasmanian Government, 2021b). Each year the scallop fishery undergoes surveys to inform harvest status. When open, peak catch and effort occurs between winter and spring/early summer (with the fishery closing 31 December) and harvesting done by benthic dredging in waters greater than 20 m deep (Tasmanian Government, 2021b, Semmens et al., 2020). The total allowable catch for the 2022 season is 3,495 tonnes (Tasmanian Government, 2022a). Historically, Commercial Scallops have mainly been for the domestic market, although export did grow substantially in 2005, particularly to France (Semmens et al., 2020).

3.4. Recreational harvest

Although BMS may be found in many areas around Tasmania, recreational harvesting for human consumption is not recommended from some areas or at certain times of the year. The Tasmanian Shellfish Market Access Programs (ShellMAP) Regulatory Services do not routinely monitor non-

commercial shellfish growing areas and recreational harvesters should adhere to standing and specific advisories from the Director of Public Health (Tasmanian Government, 2020, Tasmanian Government, 2021a). Recreational shellfish collection of wild caught BMS is permitted for clams, cockles, pipis, wedge shells (type of small pipi), mussels, Pacific Oysters and Native Oysters with bag limits specified for all except Pacific Oysters (Tasmanian Government, 2019b).

Recreational scallop (Commercial, Queen and Doughboy) fishing in Tasmania requires a licence, with a bag limit of 50 scallops per day and only open from April to end of July (Tasmanian Government, 2022c, Tasmanian Government, 2022d). In contrast commercial scallop fishing in 2022 was open from end of June to end of December (Tasmanian Government, 2022b). However, fisheries may be closed at spawning to protect stock or limit the catch for a fishery. Commercial Scallops have a protracted spawning season involving several partial spawning events with spawning lasting 5–6 months during spring and summer. After external fertilisation, larvae remain in the water column for 30 days before settling on fine to coarse sand (generally without organic sediment) forming beds (Semmens et al., 2020). Currently, recreational/indigenous scallop fishing is allowed by dive in all areas except the D'Entrecasteaux Channel, Tasmania. The recreational scallop dive fishery elsewhere around the state has supported open seasons each year from 2012 with approximately 13,000 and 130,000 scallops landed in the 2013 and 2018 recreational seasons respectively, primarily from the central east coast of Tasmania (Semmens et al., 2020).

The total estimated catch of recreational BMS in Tasmania in 2017-18 was 129,670 scallops (*Pectinidae* spp.) and 806,505 other bivalve species including mussels (*Maccullochella peelii*) and oysters (*Ostreidae* and *Pteriidae* spp.) (Lyle et al., 2019). Most recreational shellfish fishing is done inshore with a small percentage of recreational bivalve fishing done from estuarine environments (Lyle et al., 2019).

4. *Vibrio* in oysters and mussels

4.1. Environmental concentrations

4.1.1. *Vibrio parahaemolyticus*

In the aquatic environment, *Vibrio* can be concentrated in BMS due to their filter feeding behaviour. Recent modelling studies suggested sea surface temperature (SST) variation, wind speed and salinity are strong factors impacting the background concentration of *Vibrio* in oysters (Ndraha et al., 2021, King et al., 2021). In a review of various of Pacific Oyster *Vibrio* surveys undertaken in New Zealand between 2008 and 2017, it was noted that salinity and SST explained <50% of the variability in *V. parahaemolyticus* concentration; indicating other environmental or biological factors were also at play (King et al., 2021). Correlation of *Vibrio* concentrations (*V. parahaemolyticus* or *V. vulnificus*) in shellfish with rainfall events were found to be weak in the New Zealand studies (King et al., 2021).

The levels of environmental *Vibrio* in BMS vary with climatic conditions. For example, oysters harvested in the tropical or subtropical regions generally contain higher levels of *Vibrio* than oysters harvested from temperate regions (Ndraha et al., 2020). However, there are reports showing high concentrations of *V. parahaemolyticus* in the BMS collected from temperate regions of New Zealand (Kirs et al., 2011, Cruz et al., 2015b) and the USA (Jones et al., 2014). In temperate regions, SST is a driving factor for *Vibrio* in BMS, whereas in tropical regions where SST does not vary greatly, the driving factors are complex and are attributed largely to salinity (Ndraha et al., 2020). The level of *Vibrio* in BMS may also vary with shellfish type and culture conditions. For instance, wild oysters contain more *V. parahaemolyticus* than aqua-cultured oysters (Jones, 2017) and oysters grown on the sediments have higher levels of *Vibrio* than those grown in water suspension well above the sediment (Cole et al., 2015, Cruz et al., 2020, Scro et al., 2022). Intertidal harvesting has been found to increase levels of *Vibrio* in BMS (Jones et al., 2016).

In New Zealand it was found that production practice (i.e. floating, subtidal or intertidal at different depths) did not significantly impact levels of *Vibrio* in Pacific Oysters (Cruz et al., 2020). Storm events such as high winds and large volumes of precipitation may also impact the concentrations of *Vibrio* in oysters and water columns (Shaw et al., 2014).

Recent studies in temperate growing regions of Australia (Madigan et al., 2017) and New Zealand (King et al., 2021) have indicated a high prevalence of *V. parahaemolyticus* in shellfish. *Vibrio parahaemolyticus* has been studied in Australian oysters since the 1970's using a series of snapshot or point in time surveys, with a variety of diagnostic methodologies, making direct comparison of results among studies difficult (Madigan et al., 2017, Madigan et al., 2007, Lewis et al., 2003, Tamplin et al., 2011, Desmarchelier, 1978, Eyles et al., 1985). Nevertheless, total *V. parahaemolyticus* and strains containing the pathogenicity determinants *tdh* or *trh* have been detected in both Pacific and Sydney Rock Oysters from various growing regions in New South Wales, South Australia and Tasmania. In the 1970s, *V. parahaemolyticus* prevalence was reported to range from 20-68% in Sydney Rock Oysters from New South Wales, with levels showing an association with water temperature; *V. parahaemolyticus* was not detected when water temperatures were <16ºC. (Desmarchelier, 1978, Lewis et al., 2003). Eyles et al. (1985) reported levels of *V. parahaemolyticus* in natural and depurated Sydney Rock Oysters from New South Wales to range from 2.2 to 110 MPN/g and suggested that depuration was not sufficient for removing *V. parahaemolyticus* from shellfish. A snapshot survey (n=40 samples) for total and pathogenic *V. parahaemolyticus* in Sydney Rock Oyster (New South Wales) and Pacific Oysters (South Australia and Tasmania) was undertaken in Autumn of 2002 (Lewis et al., 2003). This was the first study to investigate presence of pathogenic strains using colony hybridisation with the *tdh* gene as a probe. Total *V. parahaemolyticus* was detected in 80% (n=16/20) of oysters from New South Wales, 60% (n=6/10) from Tasmania and 20% (n=2/10) from South Australia. Oysters from New South Wales had been depurated prior to testing, but still resulted in a high prevalence. Maximum levels of *V. parahaemolyticus* of 2,000 CFU/g were detected; although it is unclear if temperature control of samples was maintained during transport to the testing laboratory, which on occasion took up to 4 days (Lewis et al., 2003). *Vibrio parahaemolyticus* containing the *tdh* gene were detected in 17.5% of samples tested from all three states (n=7/40), with maximum levels ranging from 200-250 CFU/g. A single point in time survey of Pacific Oysters (n=25 samples) from Port Douglas, South Australia in December 2006 identified total *V. parahaemolyticus* in 16% of samples with levels of 2,800 to 13,000 CFU/g detected (Madigan et al., 2007). Pathogenicity determinants were detected in *V. parahaemolyticus* in some samples at levels <10 CFU/g; a prevalence of 12% of *V. parahaemolyticus trh*+ isolates were detected with no *tdh*+ isolates detected (Madigan et al., 2007). Levels of *V. parahaemolyticus* in Pacific and Sydney Rock Oysters harvested from Port Stevens, New South Wales in autumn 2009 and summer 2010 were reported to range from 2.4 to 4.0 $\log_{10}$ MPN/g (251 - 10,000 MPN/g) (Tamplin et al., 2011). However, transport to the testing laboratory was longer than 24 hrs and samples were not refrigerated, or temperature logged during transport. Madigan et al. (2017) reported 100% prevalence of *V. parahaemolyticus* in Pacific Oysters from Moulting Bay, Tasmania in the autumn of 2016, although this was a limited temporal survey of one growing area (n=30 samples). Levels of *V. parahaemolyticus* detected ranged from 0.9-16,000 MPN/g, although some samples might have been temperature abused in transit to the testing laboratory (Madigan et al., 2017). Pathogenicity determinants were detected at low levels in isolates from these samples; *tdh*+ at 20% prevalence (0.3-4 MPN/g) and *trh*+ at 17% prevalence (0.3-9 MPN/g).

In New Zealand, *V. parahaemolyticus* prevalence of 85% has been reported in Pacific Oysters sampled from 8 coastal regions between 2008 and 2017 (n=547 samples) (King et al., 2021). Prevalence and levels varied with geographic region investigated, with overall mean levels of *V. parahaemolyticus* ranging from 1 to 4,457 MPN/g. A New Zealand study conducted between 2009 to 2012 reported a *V. parahaemolyticus* prevalence of 81% (n=235 samples) in Pacific Oysters and 34% (n=55 samples) in Greenshell Mussels (*Perna canaliculus*) (Cruz et al., 2015a). It was noted that the levels of *V.*

parahaemolyticus in samples exceeded 1,000 MPN/g only when sea water temperatures were >19ºC. Geographic and shellfish species related variability in *V. parahaemolyticus* levels was observed; with no oyster samples from the South Island testing positive, and only low prevalence and levels detected from mussels sampled on the South Island. In general *V. parahaemolyticus* levels in oysters ranged from 3,600 to 2,400 MPN/g, while in mussels they ranged from 0.36 to 95.4 MPN/g (Cruz et al., 2015a). Only 1.2% of the Pacific Oyster samples from the North Island of New Zealand were *tdh*+; none were *trh*+. Kris et al (2011) surveyed Pacific Oysters (n=58 samples) during the summer/autumn of 2008-2009 from six oyster growing areas on the North Island of New Zealand and detected a *V. parahaemolyticus* prevalence of 94.8%, with a geometric mean concentration of 99.3 MPN/g (range <3 MPN/g to 1,500 MPN/g).

In a USA study, Mudoh et al. (2014) estimated a background concentration of *V. parahaemolyticus* of 3.5 log CFU/g (about 3,162 CFU/g) in Eastern Oysters (*Crassostrea virginica*) when harvest water temperature and salinity were 22.8 ± 2.71 °C and 21.47 ± 5.11 ppt, respectively. In another USA study Parveen et al. (2013) reported background levels of *V. parahaemolyticus* of approximately 2.1 to 2.8 log CFU/g (115 to 631 CFU/g) and 3.88 log CFU/g (7,585 CFU/g) respectively in Eastern Oysters and Asian Oysters (*Crassostrea ariakensis*) harvested during the warmer months. Several studies suggest *V. parahaemolyticus* die or become inactive at temperatures <10ºC (Kirs et al., 2011, Parveen et al., 2013), although there is also some evidence of their growth at such lower temperatures (Ndraha et al., 2020). Under cold and nutrient deficient conditions, *V. parahaemolyticus* may enter a VBNC state, when their metabolic activity slows down and often remain undetected (Yoon and Lee, 2022, Mizunoe et al., 2000).

4.1.2. *Vibrio vulnificus*

In Australia *V. vulnificus* was detected in Pacific Oysters sampled from Moulting Bay, Tasmania during summer/autumn of 2016; prevalence of 53% (n= 16/30) and levels ranging from 0.4 - 600 MPN/g, with no clear correlation to harvest water temperature (Madigan et al., 2017). Prior to this, *V. vulnificus* was either not investigated in Australian shellfish or not detected in limited investigations. In 2006, oysters (n=25 samples) were investigated from South Australia in a single sampling event, but *V. vulnificus* was not detected. (Madigan et al., 2007).

In New Zealand *V. vulnificus* has been detected in 17.2% (n=10/58) of Pacific Oysters sampled during summer 2008/09 from the North Island, with a mean concentration of 7.4 MPN/g, (Kirs et al., 2011). Prior to 2008 *V. vulnificus* was not investigated in New Zealand shellfish (King et al., 2021). *Vibrio* surveys undertaken in New Zealand between 2008-2017 have indicated a *V. vulnificus* prevalence of 15.3% (n=106/693) with a mean concentrations of 0.5 to 416 MPN/g depending on the region surveyed (King et al., 2021). The highest concentration of *V. vulnificus* detected was 23,800 MPN/g (King et al., 2021).

In the USA, approximately 0.8 to 4.4 log MPN/g (6 to 25,118 MPN/g) and approximately 3.2 log MPN/g (1,412 MPN/g) *V. vulnificus* were reported in Eastern Oysters and Asian Oysters, respectively; these were harvested in the warmer months with concentrations declining to below the limit of detection in the cooler months (DaSilva et al., 2012). Another USA study reported approximately 10,000 CFU/g *V. vulnificus* in freshly harvested Eastern Oysters from warm and low salinity waters (27 ± 1.7ºC and 9.1 ± 2.7 ppt) (Lorca et al., 2001).

4.2. Post-harvest concentrations

4.2.1. *Vibrio parahaemolyticus*

The indigenous microbiota (including *Vibrio*) present within BMS may grow during post-harvest treatments, including during storage and transport, causing spoilage of the product and possible foodborne bacterial illness (Madigan et al., 2014). Elevation in the concentration of *Vibrio* spp. in BMS

may occur during transport and storage if temperature is not appropriately controlled. To control post-harvest growth of bacteria in shellfish, the ASQAP manual recommends to reduce the shell stock temperature to ≤10 ℃ within 24 hours of harvest unless there is scientific evidence supporting the lack of bacterial pathogen proliferation at a higher temperature (ASQAAC, 2022). The storage temperature requirement set out in the New South Wales "Shellfish Industry Manual" are oyster species specific (NSW Food Authority, 2018). Pacific Oysters, Agassi Oysters and all other shellfish are to be refrigerated ≤10°C within 24 hours of harvest or depuration, while Sydney Rock Oysters harvested at ≤25°C or less are to be refrigerated to ≤10°C within 24 hours; if harvested at ≤21°C or less, they are to be refrigerated within 72 hours or following depuration (NSW Food Authority, 2018).

Background levels of *V. parahaemolyticus* (3.5 log CFU/gm; 3,162 CFU/g) in Eastern Oysters have been reported to triple within 3 days when stored at 20ºC, but did not significantly change when oysters were stored at 5ºC and 10ºC for 3 days (Mudoh et al., 2014). Background concentrations of *V. parahaemolyticus* in Eastern and Asian Oysters have been reported to increase 3-4 log CFU/g within 3 days of storage at 20 to 30ºC (Parveen et al., 2013). When stored at 5ºC for 10-18 days *V. parahaemolyticus* levels in Eastern and Asian Oysters were shown to decrease to levels <10 CFU/g (limit of detection of the utilised diagnostic assay) from the initial naturally contaminated levels of 2.13-2.33 log CFU/g. In the same study storage at 10ºC decreased *V. parahaemolyticus* levels on average 1.12 log CFU/g over 14-21 days (Parveen et al., 2013). By contrast unopened Sydney Rock Oysters containing backgrounds level of *V. parahaemolyticus* of 22 – 110 MPN/gm, did not significantly change when stored at 15°C and 30°C for 2 - 7 days (Eyles et al., 1985). A significant increase in *Vibrio* levels from 70 MPN/g to 23,000 MPN/g was observed when Sydney Rock Oysters were stored at 37°C for 1 day and levels rose further to 150,000 MPN/g following 2 days of storage (Eyles et al., 1985). The same study also reported *V. parahaemolyticus* growth in shucked Sydney Rock Oysters and *Vibrio* levels increased when stored at 15°C and above, but decreased when stored at 10°C (Eyles et al., 1985). *Vibrio parahaemolyticus* increased significantly from 50 MPN/g to 50,000 MPN/g, 1,400,000 MPN/g and 1,500,000 MPN/g when shucked oysters were stored at 15°C (2 days), 30°C (1 day) and 37°C (1 day), respectively (Eyles et al., 1985). Similar observations were reported by Fernandez-Piquer (2011) with background levels of *V. parahaemolyticus* (approximately 2.4 to 3.8 log MPN/g or 251 to 6,309 MPN/g) in Sydney Rock Oysters remaining unchanged at 15 – 28°C. However, when Pacific Oysters were stored at 23°C for 60 hrs and 28°C for 40 hrs, background levels of *V. parahaemolyticus* (2.4 log MPN/g or 251 MPN/g) significantly increased to 4.4 log MPN/g (25,118 MPN/g) and 4.0 log MPN/g (10,000 MPN/g), respectively (Fernandez-Piquer, 2011). A hypothesis for the discrepancy in growth was that some oyster species, such as Sydney Rock Oysters, may contain antibacterial factors which may inhibit the growth of *Vibrio* during storage (Fernandez-Piquer, 2011). Hence, post-harvest growth of *Vibrio* in oysters may not be solely dependent on storage temperature, but may also depend on oyster species, and background levels and strain types of *Vibrio*.

Fernandez-Piquer (2011) also reported different growth patterns for a cocktail of *V. parahaemolyticus* strains when artificially injected into both live Pacific Oysters and Sydney Rock Oysters and subsequently stored at various temperatures (Fernandez-Piquer, 2011). In Pacific Oysters artificially spiked with approximately 3.4 log CFU/g (2,511 CFU/g) *V. parahaemolyticus*, no increase in bacterial growth was observed when stored between 4°C and 15°C for 3 to 7 days (Fernandez-Piquer, 2011). However, significant increases in *V. parahaemolyticus* levels were observed with storage at temperatures of 18°C to 30°C. The highest concentration of approximately 7.4 log CFU/g (25,118,864 CFU/g) was recorded at 26°C following 3 days of storage (Fernandez-Piquer, 2011). In comparison *V. parahaemolyticus* didn't grow beyond spiked levels in the Sydney Rock Oysters stored at any of the same experimental temperatures (Fernandez-Piquer et al., 2011). In another study using Pacific Oyster slurry spiked with approximately 3 log CFU/g (1,000 CFU/g) of a single pathogenic strain of *V. parahaemolyticus*, growth declined to below the limit of detection when stored at 10°C and 15°C within 4 and 3 days respectively, but slightly increased when held at 20°C for 2 days (Yoon et al., 2008). Liu et al

(2009) investigated the effect of flash freezing and frozen storage on *V. parahaemolyticus* innoculatd Pacific Oysters and found that the levels of *V. parahaemolyticus* were reduced by 4.55, 4.13, and 2.53 log MPN/g after 6 months of storage at -10°C, -20°C, and -30°C, respectively (Liu et al., 2009).

4.2.2. *Vibrio vulnificus*

Post-harvest concentrations of *V. vulnificus* in BMS are also impacted by storage temperature. Background levels (0.78 to 4.4 log CFU/g; 6 to 25,118 CFU/g) of *V. vulnificus* in Eastern Oysters from the USA decreased to non-detectable levels when stored at 5 to 10°C for 18 days, but increased significantly by approximately 1 log following 1 day of storage at 15 to 30°C (DaSilva et al., 2012). Another USA study with Eastern Oysters also reported a >1 log increase above that of background levels (10,000 CFU/g) of *V. vulnificus* following 1 day of storage at 21°C with levels remaining elevated (>100,000 CFU/g) for 10 days (Lorca et al., 2001). A one to two log reduction of *V. vulnificus* were observed in Eastern and Pacific Oysters stored at 0.5, 8 and 22°C when whole oysters were injected with approximately 11,000 CFU/g of *V. vulnificus* (Kaysner et al., 1989). In another study of Eastern Oysters, which had been bioaccumulated with approximately 100,000 MPN/g *V. vulnificus*, a rapid decline of >1 log was observed when oysters were stored at 0, 2 and 4°C, but levels increased by >2 log when stored at 30°C (Kaspar and Tamplin, 1993a). An approximate 4 log reduction *was observed* in Eastern Oysters injected with 100,000,0 CFU/g of *V. vulnificus*, when stored at -20°C (Parker et al., 1994).

5. Exposure assessment

5.1. Tasmanian *Vibrio* survey

Eleven Tasmanian shellfish growing areas were included in the *Vibrio* survey which covered three summer/autumn (November to April) and two winter/spring (May to October) sampling periods between January 2020 and end of April 2022. All growing areas selected for the survey were export approved for oyster or mussel production and represented a combination of production systems including estuarine, intertidal, subtidal, intertidal and oceanic. The production areas selected were Duck Bay, Moulting Bay, Great Swanport, Great Oyster Bay, Little Swanport, Boomer Bay, Boomer Bay East, Pitt Water, Pipe Clay Lagoon, Great Bay and Fleurtys Point (**Figure 1**). Pacific Oysters were sampled from all 11 harvest areas. Blue Mussels were also sampled in Great Swanport. Some of these harvest areas had high tidal flows while other areas were less impacted by tide. Some areas had large or multiple river inputs (Great Swanport, Great Oyster Bay, Moulting Bay, Little Swanport, Duck Bay, Pitt Water and Fluerteys Point) while others had none (Boomer Bay and Boomer Bay East, Pipe Clay Lagoon and Great Bay).

Shellfish were collected and tested for *Vibrio* on a fortnightly basis in the summer/autumn sampling periods and monthly in the winter/spring sampling periods. Total *V. parahaemolyticus*, *tdh* and *trh* positive *V. parahaemolyticus* and total *V. vulnificus* were enumerated using a combination of a 3-tube MPN followed by qPCR confirmation of turbid tubes (Kinsey et al., 2015, Nordstrom et al., 2007). *Vibrio parahaemolyticus* confirmation was done using the *tlh* gene and *V. vulnificus* confirmation was done using the *vvh* gene. Enumeration of *V. parahaemolyticus* containing the potential pathogenicity determinants *tdh* and *trh* were also done using the MPN qPCR method on turbid tubes having tested positive for total *V. parahaemolyticus*. The method used was a modification of methods by Nordstrom et al (2007) and Kinsley et al (2015).

Environmental data was also collected during the survey. This included sampling and growing water temperature, maximum air temperature on day of harvest, growing water salinity, rainfall 1, 2, 3 and 7 days before sampling and maximum river flow on day of sampling for any rivers identified for the particular harvest area from the sanitary survey (further details can be found in the individual growing

area survey data in the appendices). Temperature data loggers (ibuttons) were deployed to all harvest areas to monitor harvest water temperature prior to sampling and to confirm that samples were not temperature abused during transit to the Public Health Laboratory in Hobart. The definition of temperature abuse was based on the parameters defined in the Tasmanian *Vibrio* control plan (Tasmanian Government, 2019a). There it is stated that from 1st November until 30th April the time from harvest into the cool chain (<10°C) must not be greater than 12 hours. When ambient air temperature is greater than 30°C, the time from harvest into the cool chain must not be greater than 7 hours. When water temperature at the depth where oysters are harvested is greater than 19°C, the time from harvest into cool chain must not be greater than 7 hours. For our purposes we used the most stringent of these requirements to define temperature abused (<10°C within 7 hours of harvest). Samples deemed temperature abused were excluded from analysis. Statistical analysis determined which environmental predictors were significantly associated with *V. parahaemolyticus* or *V. vulnificus* (if applicable) in each growing area, and which predictor variables contributed to the development of the best linear predictive models for *V. parahaemolyticus* risk and *V. vulnificus* (see Appendices 1-12).

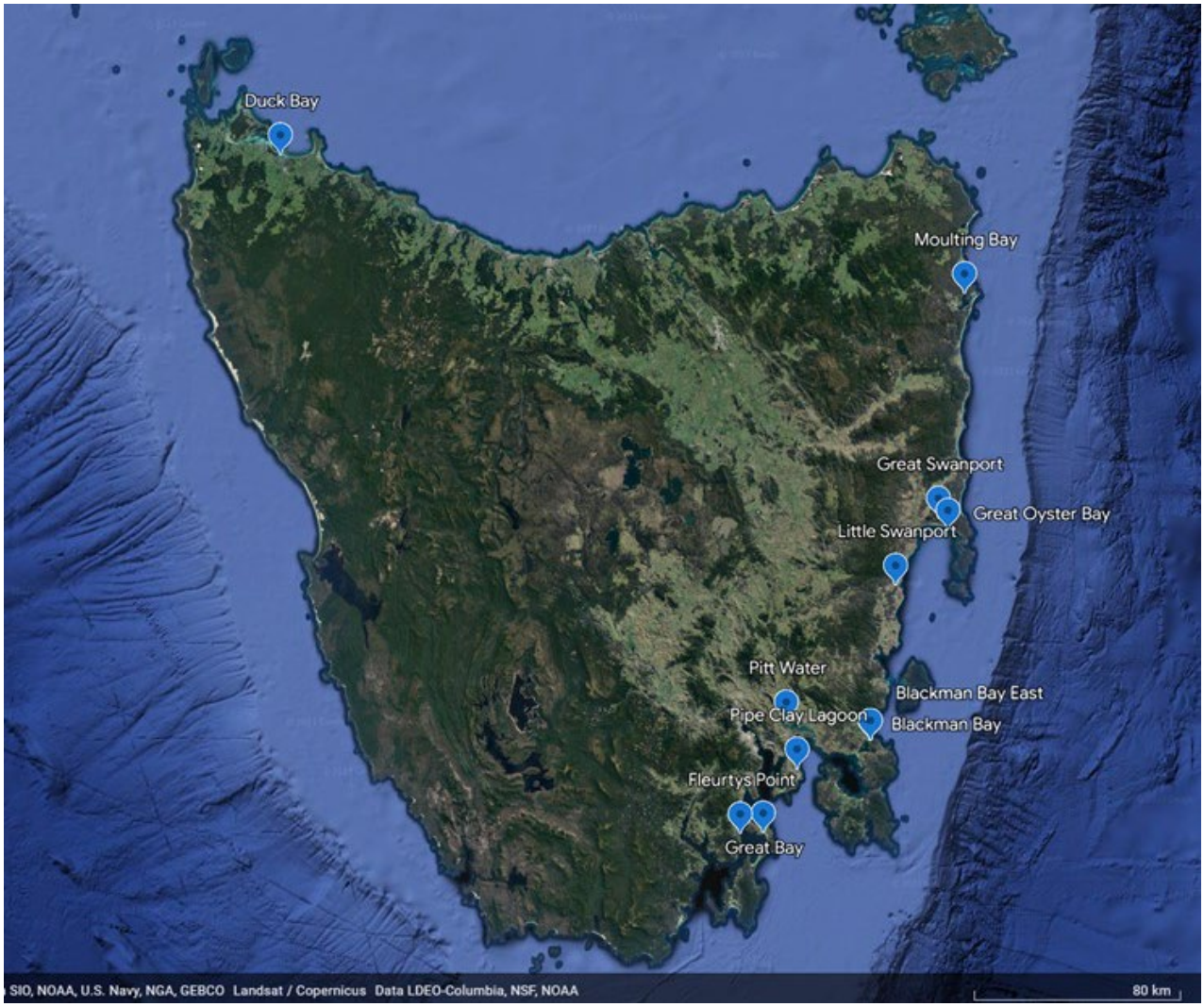

Figure 1: Map of Tasmania showing shellfish production areas sampled in the *Vibrio* survey. The surveyed growing areas belong to the following Regions: North West (Duck Bay), Moulting Bay (Moulting Bay), Upper East Coast (Great Swanport, Great Oyster Bay and Little Swanport), Mid-East Coast (Boomer Bay and Boomer Bay East), South-East Region (Pitt Water and Pipe Clay Lagoon) and Bruny Region (Great Bay and Fleurtys Point).

Results of the *Vibrio* survey are shown in **Table 2** for *V. parahaemolyticus* and **Table 3** for *V. vulnificus.*

Strong seasonal patterns were observed in *V. parahaemolyticus* prevalence state-wide, with higher prevalence consistently observed in the summer/autumn sampling periods as compared with the winter/spring sampling periods. During the summer/autumn sampling period a higher prevalence of *V. parahaemolyticus* was found in shellfish harvested from areas in the north-west, Moulting Bay and upper east coast as compared with the mid-east coast and south-east and Bruny regions (**Figure 2)**. Maximum levels of *V. parahaemolyticus* detected in shellfish were also higher in areas from the north-west, Moulting Bay and upper east coast regions (110-1,100 MPN/g; **Table 2**). Shellfish taken from the mid-east coast and south-east and Bruny regions had maximum levels of *V. parahaemolyticus* in summer/autumn samples of 3-23 MPN/g.

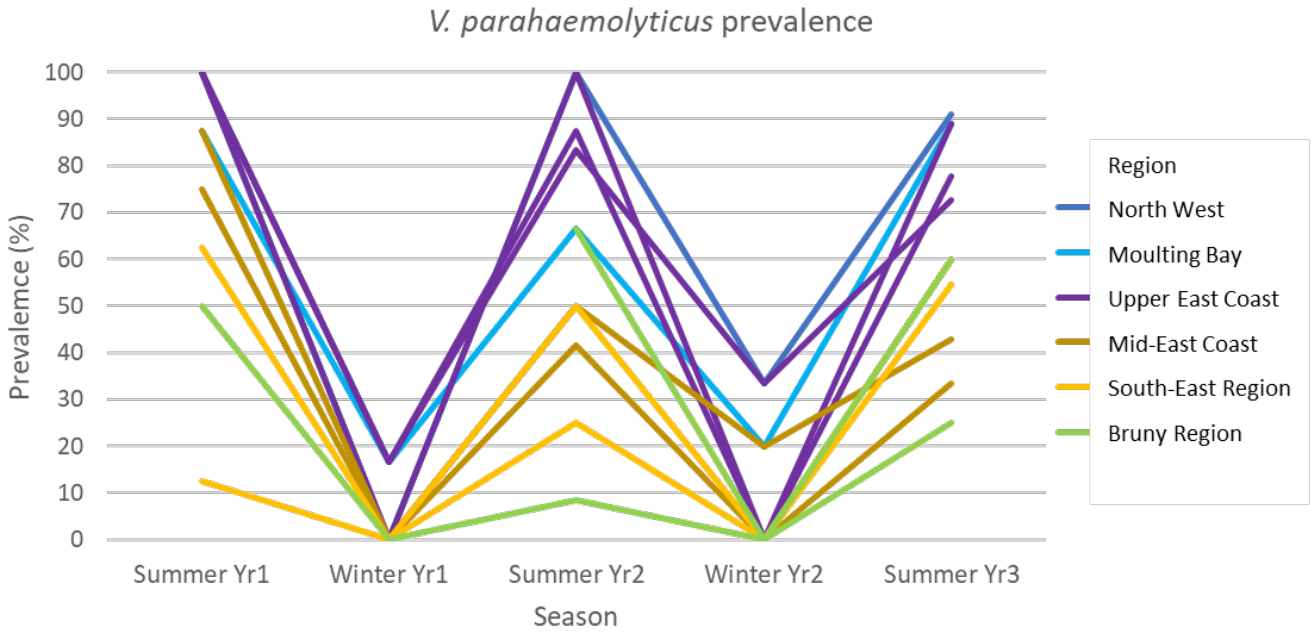

Figure 2: Seasonal and regional prevalence of *V. parahaemolyticus* in Tasmania

Air and water temperature (sampling, minimum and maximum water temperature 3 days prior to sampling or average 3-day water temperature prior to sampling) were significant predictors of *V. parahaemolyticus* risk and often highly inter-correlated (see Appendix 1-12 for individual harvest area pre-harvest predictive models). Rainfall was also a significant individual predictor of *V. parahaemolyticus* risk in Great Swanport (oysters only), Boomer Bay and Duck Bay, while salinity was a significant individual predictor only in Duck Bay. Maximum river flow was a significant predictor of *V. parahaemolyticus* risk in Duck Bay and Fluertys Point. Predictive linear models of risk were largely driven by water temperature prior to harvest in isolation, or in combination with harvest water salinity or rainfall (**Table 2**). *Vibrio parahaemolyticus* was detected in all areas when the minimum average harvest water temperature 3 days prior to sampling was at or above 11-15°C. *Vibrio parahaemolyticus* containing the pathogenicity associated *tdh* and/or *trh* genes were detected in all growing areas surveyed except Pitt Water. *Tdh*+ and/or *trh*+ *V. parahaemolyticus* were only detected during the summer/autumn surveyed periods when prevalence of *V. parahaemolyticus* was highest (**Figure 3**). Prevalence of *tdh* strains varied from 0-22% and *trh* strains varied from 0-57% in individual growing areas. In areas where *tdh*+/*trh*+ *V. parahaemolyticus* strains were detected, their presence was not consistent i.e. they were not detected in one or more of the surveyed summer/autumn periods (**Figure 3**.

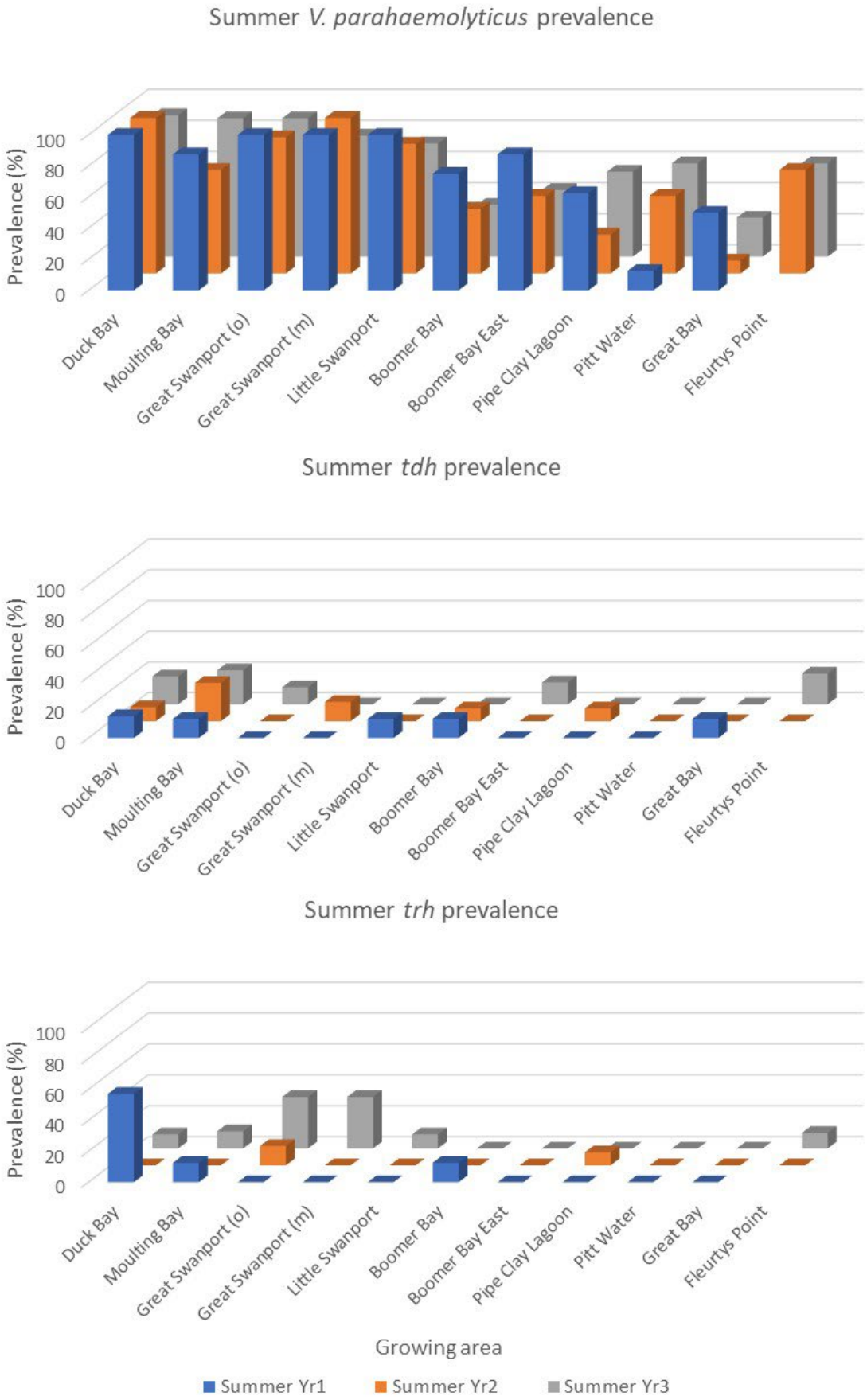

Figure 3: Regional summer prevalence of total and pathogenic (*tdh*+ and *trh*+) *V. parahaemolyticus*.

Vibrio vulnificus was only detected in the summer/autumn sampling periods with a low prevalence in most surveyed harvest areas, except Duck Bay and Boomer Bay where it wasn't detected (**Figure 4**). Great Swanport was the only harvest area (oysters and mussels) which showed a higher prevalence, particularly in the third summer/autumn sampling period. In areas where *V. vulnificus* was detected, levels were generally low (<1 MPN/g; **Table 3**). However, in Great Swanport, levels of 35 MPN/g were detected in oysters and 460 MPN/g were detected in mussels in the last summer/autumn sampling period. Individually, maximum river flow of the Apsley River was the only significant predictor of *V. vulnificus* risk in Great Swanport for both oysters and mussels. The best linear predictive model for *V. vulnificus* risk in this area was determined by maximum river flow of the Apsley River and 1-day rainfall prior to sampling (**Table 3**).

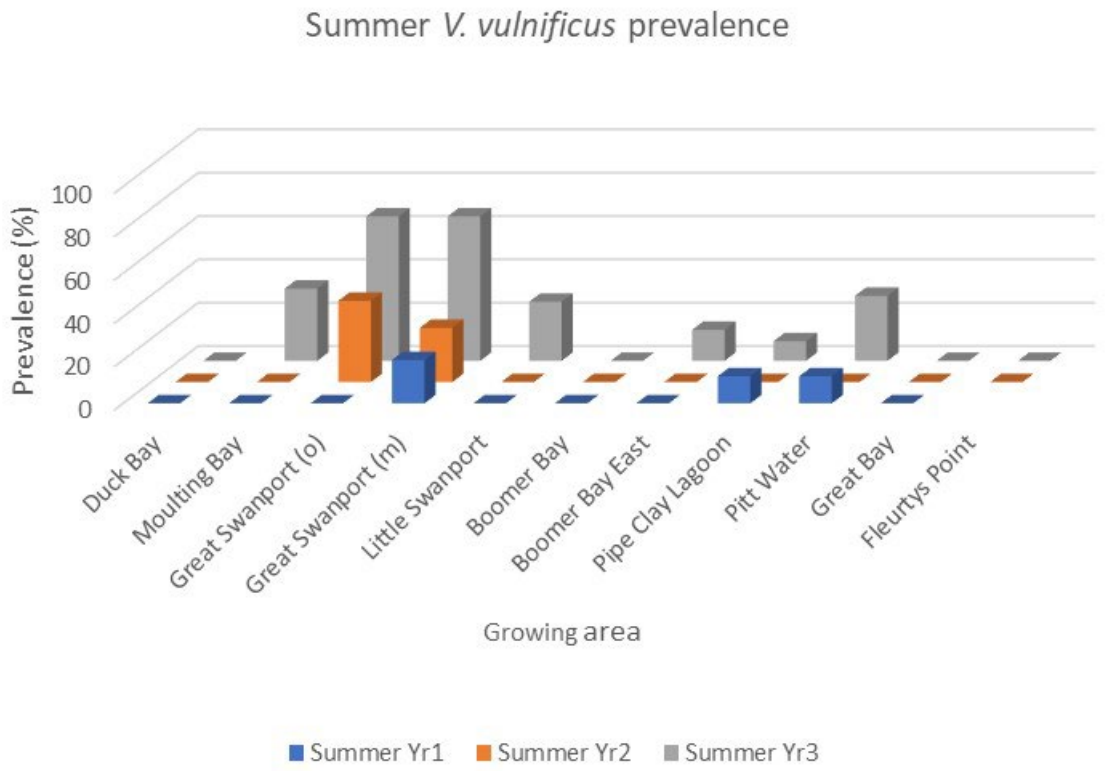

Figure 4: Regional summer prevalence of *V. vulnificus*.

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Table 2: Summary of *V. parahaemolyticus* (*Vp*) prevalence and levels in Tasmanian shellfish growing areas and links with environmental factors. See Appendices 1 to 12 for detailed results and modelling.

Region and growing area	Shellfish (n=non abused samples)	Summer *Vp* prevalence (%) in Yr1;Yr2;Yr3	Summer *tdh*+ Vp prevalence (%) in Yr1;Yr2:Yr3	Summer *trh*+ Vp prevalence (%) in Yr1;Yr2;Yr3	Max *Vp* detected (MPN/g)	Winter *Vp* prevalence [1] (%) in Yr1;Yr2	Drivers for *Vp* risk: Primary (Secondary) and correlation co-efficient	Min Av H_20 temp linked with *Vp* (°C)[2]
North West								
Duck Bay	Oysters (n=21)	100;100;91	14;9;18	57;0;9	460	0;33	Max. water temperature 3-d prior to sampling. R^2=0.69.	12
Moulting Bay								
Moulting Bay	Oysters (n=30)	88;67;89	13;25;22	13;0;11	110	17;20	3-d average water temperature. R^2=0.46.	15
Upper East Coast								
Great Swanport	Oysters (n=25)	100;88;89	0;0;11	0;13;33	1100	17;0	Min. water temperature 3-d prior to sampling (salinity). R^2=0.48.	11
Great Swanport	Mussels (n=25)	100;100;78	0;13;0	0;0;33	460	0;0	Min. water temperature 3-d prior to sampling (salinity). R^2=0.50.	12
Great Oyster Bay [3]	Oysters (n=10)	60;NA;NA	0;NA;NA	0:NA;NA	0.3	0;NA	NA	NA
Little Swanport	Oysters (n=41)	100;83;73	13;0;0	0;0;9	460	17;33	Max. daily air temperature (2-d rainfall). R^2=0.36.	13
Mid-East Coast								
Boomer Bay	Oysters (n=31)	75;42;33	13;8;0	13;0;0	23	0;0	3-d average water temperature (7-d rainfall). R^2=0.39.	13
Boomer Bay East	Oysters (n=25)	88;50;43	0;0;14	0;0;0	9.2	0;20	Min. water temperature 3-d prior to sampling. R^2=0.26.	13
South-East Region								
Pitt Water	Oysters (n=40)	13;50;60	0;0;0	0;0;0	16	0;0	Min. water temperature 3-d prior to sampling. R^2=0.27.	12
Pipe Clay Lagoon	Oysters (n=43)	63;25;55	13;8;0	0;8;0	3.6	0;0	7-d rainfall (Sampling water temperature). R^2= 0.29.	12
Bruny Region								
Great Bay	Oysters (n=40)	50;8;25	13;0;0	0;0;0	11	0;0	Max. daily air temperature; with a low coefficient of determination (R^2=0.11).	15
Fleurtys Point	Oysters (n=21)	NA;67;60	NA;0;20	NA;0;10	3	NA;0	Min. water temperature 3-d prior to sampling. R^2=0.44.	15

[1] *tdh*+ and/or *trh*+ *V. parahaemolyticus* strains were not detected in the winter/spring sampling periods.

[2] Minimum average harvest water temperature 3 days before sampling for shellfish testing positive for *V. parahaemolyticus*.

[3] Too few samples submitted to determine seasonal prevalence or relationship with environmental drivers.

Table 3: Summary of *V. vulnificus* prevalence and levels in Tasmanian shellfish growing areas and links with environmental factors. See Appendices 1 to 12 for detailed results and modelling.

Growing area	Shellfish	Summer *V. vulnificus* prevalence (%) in Yr 1;Yr2;Yr3	Maximum *V. vulnificus* detected (MPN/g)	Drivers for *V. vulnificus* risk
North West				
Duck Bay	Oysters	0;0;0	Not detected	Not applicable
Moulting Bay				
Moulting Bay	Oysters	0;0;33	0.94	Unable to be determined due to low prevalence
Upper East Coast				
Great Swanport	Oysters	0;38;67	35	Max river flow of Apsley River and 1 day rainfall
Great Swanport	Mussels	20;25;67	460	Max river flow Apsley River and 1 day rainfall
Great Oyster Bay[1]	Oysters	20 (n=1/5);NA;NA	0.3	Unable to be determined
Little Swanport	Oysters	0;0;27	0.72	Unable to be determined due to low prevalence
Mid-East Coast				
Boomer Bay	Oysters	0;0;0	Not detected	Not applicable
Boomer Bay East	Oysters	0;0;14	0.36	Unable to be determined due to low prevalence
South-East Region				
Pitt Water	Oyster	13;0;30	0.73	Not applicable
Pipe Clay Lagoon	Oysters	13;0;9	0.36	Unable to be determined due to low prevalence
Bruny Region				
Great Bay	Oysters	0;0;0	Not detected	Not applicable
Fleurtys Point	Oysters	NA;0;0	Not detected	Not applicable

[1] Too few samples submitted to determine seasonal prevalence or relationship with environmental drivers.

5.1.1. Strain typing of environmental *V. parahaemolyticus* from Tasmania

During the *Vibrio* survey, putative *Vibrio* colonies were also isolated for further genomic characterisation. As part of the MPN qPCR testing undertaken, the Public Health Laboratory would streak random turbid MPN tubes onto TCBS and select putative *V. parahaemolyticus* (green) colonies for further confirmation and classification by SARDI. One hundred and eighty-four pure bacterial colonies were isolated and sent to SARDI for *V. parahaemolyticus* confirmation by qPCR. Sixty-six percent of the bacterial colonies were identified as *V. parahaemolyticus* (n=121) by the presence of the *tlh* gene. The remaining 34% (n=63) of putative *Vibrio* isolates were not characterised further. None of the identified *V. parahaemolyticus* cultures contained the *tdh* or *trh* genes.

Select *V. parahaemolyticus* (n=48) isolates were further characterised by WGS MLST by SA Pathology (**Table 4**). In total 20 *V. parahaemolyticus* ST types were identified during this survey. Seven were unique ST types identified as part of this study (ST3263-ST3267, ST 3359 and ST3360). Most of the other ST types detected had previously only been reported in environmental (n=10) samples, but three ST types had previously been reported in both clinical and environmental samples (ST12, ST141 and ST396) within the PubMLST database (Jolley et al., 2018). However, peer reviewed scientifically published data regarding clinical reports of these three ST types is scare.

Table 4: Genomic (MLST) typing of Tasmanian environmental *V. parahaemolyticus* isolates.

MLST	No. of detections	Growing area	Years detected	Unique ST	Clinical/Environmental as report in PubMLST[1]
12	4	Duck Bay	2020, 2021	No	Clinical & Environmental
57	1	Pitt Water	2022	No	Environmental
141	1	Boomer Bay	2020	No	Clinical & Environmental
347	11	Duck Bay (n=1) Great Swanport (n=2) Little Swanport (n=5, Boomer Bay East (n=2) Pitt Water (n=1)	2022 2019, 2021 2020 2020 2022	No	Environmental
396	3	Moulting Bay (n=1) Great Swanport (n=1) Fleurtys Point (n=1)	2021 2020 2022	No	Clinical & Environmental
423	2	Boomer Bay	2021	No	Environmental
495	2	Great Swanport (n=1) Pipe Clay Lagoon (n=1)	2022 2020	No	Environmental
645	5	Duck Bay (n=1) Great Swanport (n=2) Little Swanport (n=2)	2022 2021, 2022 2021, 2022	No	Environmental
1061	1	Duck Bay	2020	No	Environmental
1357	2	Duck Bay (n=1) Little Swanport (n=1)	2021 2020	No	Environmental
2955	2	Duck Bay (n=1) Great Swanport (n=1)	2022 2022	No	Environmental
3187	1	Moulting Bay	2020	No	Environmental
3201	1	Moulting Bay	2022	No	Environmental
3263	1	Great Swanport	2020	Yes	Environmental (this study)
3264	1	Moulting Bay	2020	Yes	Environmental (this study)
3265	2	Great Bay	2020, 2021	Yes	Environmental (this study)
3266	3	Little Swanport (n=2) Pipe Clay Lagoon (n=1)	2022	Yes	Environmental (this study)
3267	3	Moulting Bay (n=2) Little Swanport (n=1)	2019, 2020 2022	Yes	Environmental (this study)
3359	1	Pitt Water	2020	Yes	Environmental (this study)
3360	1	Little Swanport	2020	Yes	Environmental (this study)

[1] *Vibrio parahaemolyticus* | PubMLST

ST12 has been isolated from a clinical sample of a patient exhibiting acute diarrhoea in south-eastern China but was found to be *tdh*- and *trh*- and hence classified as non-pathogenic (Chen et al., 2016). ST12 has also been detected in environmental samples from China and associated with shrimp disease (Fu et al., 2021). ST141 (VP80-1B; *tdh*-, *trh*+, *ure*R+) has been isolated from a sediment sample in the USA in 1992 and found to belong to the same phylogenetic clade as clinical strain ST50 (EN9701121; *tdh*+, *trh*+, *ure*R+) (Turner et al., 2013). Three clinical strains of ST141 (*tdh*-, *trh*+) were isolated in Canada between 2004 and 2006 (Banerjee et al., 2014). Canadian clinical ST141 strains were also found to be related to clinical ST50 strains (*tdh*+, *trh*+). Interestingly the pandemic ST3 (*tdh*+, *trh*-) was found to be related to ST417 (*tdh*-, *trh*+) (Banerjee et al., 2014). ST50 and ST417 were two of the clinical outbreak strains identified during the 2021 South Australian oyster vibriosis outbreak. ST396 has been identified in oyster, fish and shrimp (*tdh*-, *trh*-) and ready to eat cold vegetable dishes in China (Xie et al.,

2016, Yang et al., 2017) but has not been identified in clinical samples outside of the PubMLST database.

In our survey the most common ST types detected from multiple growing areas and over several years was ST347. Previously, ST347, ST57 and ST1357 have been reported from mussels in The Netherlands (ST 347 and ST57) and clams (ST1357) from Italy (Lopatek et al., 2018). Interestingly, strains belonging to these ST types were also reported to have single or multiple antibiotic drug resistance (Lopatek et al., 2018). However 75% and 68% of *V. parahaemolyticus* isolates surveyed (n=104) showed resistance to ampicillin and streptomycin, respectively (Lopatek et al., 2018). It is not uncommon for environmental *V. parahaemolyticus* to carry antibiotic resistance genes (Xie et al., 2016, Yang et al., 2017).

5.2. *Vibrio* monitoring in Tasmania

Monitoring for *V. parahaemolyticus* in Tasmanian shellfish at production doesn't occur on a regular basis. Following the 2016 Tasmanian vibriosis outbreak the Public Health Laboratory in Hobart gained accreditation for *V. parahaemolyticus* testing using the AS5013.18 (Australian Standard, 2010). Between 2016 and 2019 the regulatory shellfish program undertook monitoring for *V. parahaemolyticus* in shellfish (predominantly oysters) during the summer/autumn months from Moulting Bay (n=272). Most sampled shellfish either did not have *V. parahaemolyticus* detected (50% had <3 MPN/g) or low levels of *V. parahaemolyticus* (49% had 3 to <100 MPN/g). One sample taken in January 2018 had levels >1,100 MPN/g. It is not known if this sample was temperature abused during transit to the Public Health Laboratory in Hobart for testing, although the normal routine was for samples to be sent on an ice pack in an esky. In 2019, limited summer/autumn monitoring for *V. parahaemolyticus* in oysters also occurred in Great Swanport (n=16; levels detected ranged from 3.6-93 MPN/g), Big Bay (n=8; levels detected ranged from 9.2-43 MPN/g) and Pipe Clay Lagoon (n=8;<3 MPN/g).

Results from the Moulting Bay *V. parahaemolyticus* monitoring program, undertaken by DPIPWE, Biosecurity Tasmania in 2017/18 underpinned the development and implementation of the Tasmanian *Vibrio* Control Plan in the summer of 2017/18 (Personal CommunicationShellMAP, 2023).

Limited testing of oysters for *V. parahaemolyticus* had occurred in research projects before and after the 2016 Tasmanian vibriosis outbreak using various methodologies. In April 2002 oysters (n=10) were tested for *V. parahaemolyticus* from Little Norfolk Bay, Dunalley, Triabunna, Pitt Water, Barilla Bay, Pipe Clay Lagoon and Little Swanport; 60% were positive for *V. parahaemolyticus* and 20% were *tdh*+ (Lewis et al., 2003). The mean total *V. parahaemolyticus* levels detected in Tasmanian oysters was 2.5±0.5 $\log_{10}$ bacteria/g with a maximum count of 2,000 bacteria/g. In February 2010, oysters were tested for *V. parahaemolyticus* from Little Swanport (n=4; 9.3 – 46 MPN/g), Moulting Bay (n=4; 1.5 – 4.3 MPN/g) and Pipe Clay Lagoon (n=3; <0.3 – 4 MPN/g) (Madigan et al., 2017). No *tdh*+ or *trh*+ *V. parahaemolyticus* strains were detected at that time. Following the 2016 vibriosis outbreak, 30 samples collected from Moulting Bay (five leases) were tested for *V. parahaemolyticus* between February and May 2016; 10% of samples had levels of <3 MPN/g, 50% of samples had levels of 3-100 MPN/g, 37% of samples had levels of >100-1,000 MPN/g and 3% of samples had levels of >10,000 MPN/g (Madigan et al., 2017). The prevalence of *V. parahaemolyticus tdh*+ and *trh*+ strains during the 2016 survey were 20% and 17%, respectively. The levels of *V. parahaemolyticus* detected by Madigan et al. (2017) in Moulting Bay oysters following the 2016 vibriosis outbreak appear higher than those observed during the ShellMAP *Vibrio* monitoring program and our current survey (maximum level of *V. parahaemolyticus* detected was 1,100 MPN/g). Madigan et al (2017) highlighted the concern that oysters may have been temperature abused in transit from the growing area to the testing laboratory in Adelaide, as no temperature loggers were used during shipping to either confirm or refute abuse.

5.3. Shellfish consumption in Australia

Specific and current information on BMS consumption in Australia is lacking. The 2011-12 Australian Health Survey broadly group seafood into one category; consumption of BMS is not delineated (ABS, 2014). The portion of Australian over 19 years old consuming fish and seafood products and dishes in general is 18.9 ± 1.2% (95% CI) based on 1 day recall. In the 2004 joint FAO/IOC/WHO "ad hoc Expert Consultation on Biotoxins in Bivalve Molluscs" report, 2% of Australians were reported to consume BMS over a 1-day period (FAO/IOC/WHO, 2004).

The highest 97.5th percentile consumption of edible shellfish for Australians is reported as 181 g for adults and 70 g for children, for a single day or single sitting (FAO/IOC/WHO, 2004). The mean daily Australian intake of all molluscan shellfish (fresh and frozen) is reported at 0.2 g for males (relative standard error of estimate at 31.2%) and 0.6 g for females (relative standard error of estimate at 24.7%) (ABS, 2014). However, it is noted that data with standard error estimates of 25-50% should be used with caution.

The general Australian population would only eat 0.5 g/day molluscan shellfish, but of those which eat shellfish, on average they would consume 79 g/day. The 50th, 90th, 95th and 97th percentiles for consumers of molluscan shellfish was 63, 146, 180, and 248 g/day, respectively as estimated from the 2011-2012 National Nutrition and Physical Activity Survey (Williamtown Contamination Expert Panel, 2015).

5.4. Tasmanian supply chains

The majority of oysters harvested in Tasmania enter the domestic market, with only 3% exported (Schrobback et al., 2020). Tasmanian supply chain models include direct sale from the producer to consumer (e.g. on-line shop, on-farm retail shop, oyster tourism or shucking events) and to the retail sector (e.g. food services or fish mongers), distribution through seafood agents, processor/wholesalers and export, with some vertically integrated corporate supply chains (Schrobback et al., 2020). Processors/wholesalers are often integrated and represent a large share of the supply chain market. Oysters may pass through two or more logistics operations and change of consignment during transport (Huddlestone, 2022). Three to 7 transactions have previously been reported in the oyster supply chain (Comiskey, 2009). The number of days from harvest to wholesale is generally 3-6 days, including arrival at an export destination (**Table 5**). At wholesale, oysters are either shucked or sold whole in the shell to retail and then to consumers. The reported shelf-life of whole Tasmanian oysters is 9 days from harvest (Huddlestone, 2022). Most oysters produced in Tasmania are sold by the retail sector interstate; 60-80% through food service (restaurants, pubs and clubs), 20-30% by fishmongers and 2-3% by domestic supermarkets (Schrobback et al., 2020).

Table 5: Transit days of harvested Tasmanian oysters to domestic wholesalers and export destination (Huddlestone, 2022).

Days in supply chain				
Victoria	New South Wales	Queensland	Western Australia	Export Markets
3	4	5-6	6	4

Detailed knowledge of Australian supply chains is limited apart from historical reports. The Tasmanian oyster supply chain is medium length on a national comparison (average 2.8 days), as compared to South Australia which is longer (average 3 days) and New South Wales which is shorter (average 1 day) (Madigan, 2008). Temperature abuse (heat) has been identified as a greater issue in the South Australian supply chain in comparison with Tasmania and New South Wales (Madigan, 2008, Tamplin et al., 2011). There are also state differences between modes of oyster storage and transport. In New

South Wales in 2008, the proportion of producers with access to chillers and using refrigerated transport is much lower than Tasmania or South Australia. This is largely due to the New South Wales temperature dispensation for Sydney Rock Oysters (Madigan, 2008).

The pattern of distribution of oysters from Tasmania, New South Wales and South Australia is also different. In New South Wales the majority of product is sold in Sydney. By contrast, in Tasmania and South Australia the proportion of interstate destinations is greater, as is the increased need for shipping via distribution centers, factors which increase the potential for temperature abuse (Madigan, 2008).

Information on the prevalence or presence of *V. parahaemolyticus* in Australian oysters at retail is limited. Madigan et al., 2007 undertook a limited survey of retail and wholesale oysters in South Australia in autumn 2006 and found *V. parahaemolyticus* was below the level of detection (<10 CFU/g) using the plaque lift hybridisation assay. However, they found that the microbiological quality of oysters at harvest did not reflect in the microbiological quality of oysters at wholesale or retail. Although, total marine *Vibrio* counts (as assessed on TCBS agar plates) did not significantly differ between freshly harvested and wholesale/retail oyster, the latter had higher total viable bacterial counts (Madigan et al., 2007).

Harvest practice and season were found to affect total *Vibrio* numbers in South Australian Pacific Oysters. Inter-tidal exposure of oysters at harvest increased the numbers of *Vibrio* and return to seawater did not immediately decrease their numbers. *Vibrio* was also found to be below the level of detection (<10 CFU/g) during the winter when the water temperature was approximately 12ºC (Madigan et al., 2007).

Peak production of Tasmanian Pacific Oysters is between March and the end of November with lower production between December and February, when diploids are spawning (Comiskey, 2009).

5.5. Control measures

Limited guidance for the control of *Vibrio* exists in the Australian Shellfish Quality Assurance Programme (ASQAP) Manual of Operations, other than the statement that shellfish intended for raw consumption should be under refrigeration at 10ºC or less within 24 hours[1] (ASQAAC, 2022). As a result, this is listed as a requirement in an oyster business's "Food Safety Management System for Live Tasmanian Farmed Bivalve Molluscs" (Tasmanian Government, 2019a) audited annually. In New South Wales, guidance for temperature control of shellfish, states that Pacific Oyster, Native Oysters and all other shellfish must be placed under refrigeration at 10°C or less within 24 hours of being harvested or completion of depuration, however there is a dispensation for Sydney Rock Oysters which states that they must be placed under temperature control at 25°C or less within 24 hours of harvest and at 21°C or less within 72 hours of harvest or if harvested for depuration, after depuration is completed (NSW Food Authority, 2018).

5.5.1. Control measures in Tasmania

In 2018, *Vibrio* Control Plans (VCP) were developed and implemented in the four Tasmanian areas identified as high-risk, due to association with *Vibrio* illnesses (Big Bay, Moulting Bay, Great Swanport and Pipe Clay Lagoon). The VCP for these areas are part of the Food Safety Management System for all businesses operating in these areas (Tasmanian Government, 2019a). They enable industry to meet their food safety objectives at market, supporting the ongoing viability of the oyster industry.

[1] Note: A higher temperature will be considered acceptable only if demonstrated, by scientifically robust evidence, that such a (higher) temperature will not support unacceptable growth of human pathogens in the shellstock (ASQAP 2022).

The VCP are based on control measures on the time from harvest to cool chain (≤10°C). Note that this is the time to enter the cool chain. The time for the core temperature of shellfish to be at temperatures of below 10°C is not specified but will depend on the capacity of the refrigeration units in the cool chain. The standard requirement in Tasmanian VCP is that BMS harvested between 1st November to 30th April must be in cool chain within 12 hours of harvest (Tasmanian Government, 2019a). However, when the air temperature is greater than 30°C or the harvest water temperature is greater than 19°C, the time from harvest to cool chain must be less than 7 hours. Oysters can be transported unrefrigerated if the harvest to cool chain time is within these limits. The time count starts from the time of harvest of the first oyster or the first basket, or for intertidal oysters, when the oysters are exposed to the air during a low tide event. Oysters must be kept in the cool chain if they are harvested the day prior to pack out. Care must be taken not to agitate the sediments during handling of the oysters, and the racks or baskets must not encounter the sediments. For direct sales from the farm, oysters must be refrigerated, and their internal temperature must be ≤10°C when sold.

Although not mandatory, the state authorities recommend other growing areas in Tasmania implement a VCP in the warmer months due to increasing risks of *Vibrio* outbreaks (Tasmanian Government, 2019a).

The control measures in the VCP are supplemented by pre-harvest, harvest and post-harvest practices listed in the industry *V. parahaemolyticus* guide (Oysters Tasmania, 2019). The pre-harvest control practices in this guide include returning the oysters to the growing area for at least two tidal cycles after land-based handling activities; moving oysters to deeper, cooler waters or moving to lower risk waters for 7 days before harvest. Harvest practices that may reduce *V. parahaemolyticus* risks and include harvesting in the early morning when temperature is cooler; harvesting as soon as the oysters are exposed to air in an intertidal area; and ensuring that the harvesting is efficiently performed to limit the exposure of the oysters to the warm air. For post-harvest temperature management, shading oysters from direct sun; good air circulation; water sprays; keeping the oysters in ice slurry; and refrigeration are recommended approaches.

When placed in a chilled vessel for transport or stored in cold storage, it is important to stack the oysters for maximum air circulation and cooling (Oysters Tasmania, 2019). Cooling in ice slurry is very efficient to rapidly reduce the internal BMS temperature, but this process can cause oyster mortality and may reduce shelf life. Therefore, suitable dip time and temperature should be determined, and the ice slurry must be replaced regularly to avoid cross contamination. To control *V. parahaemolyticus* growth within the supply chain, the industry (Oysters Tasmania, 2019) has developed some messages and materials for transporters, wholesalers, processors and retailers. Recommendations for transporters are to maintain cool chain throughout the transport, not to expose oysters to warm air drafts during transport, and to refrigerate oysters immediately after delivery. Key messages for the wholesalers and processors are to record arrival temperature and to rapidly move the stock to cold storage. Maintaining cool chain in ice or via refrigeration is the key message for the retailers.

Cooking at 65°C eliminates *V. parahaemolyticus* from the oysters. The industry (Oysters Tasmania, 2019) has also listed potential post-harvest processing steps to eliminate *V. parahaemolyticus* from the oysters. These include High Pressure Processing, Individual Quick Freezing (IQF) with extended storage and irradiation.

At the time of the development of this risk profile document, Tasmania did not have a published policy or guideline outlining the criteria for harvest area closures and re-opening in regarding oyster related vibriosis (Personal Communication Loone, 2023). The management of closures and re-opening at that time was undertaken by regulators on a case-by-case basis in conjunction with information provided by the Department of Health. The management approach at the time gave consideration to the USFDA

NSSP and “South Australian *Vibrio* Harvest Area Detection Protocol” (NSSP, 2019, Personal Communication Dowsett, 2022).

5.5.2. Control measures in South Australia

Similar VCP have been adopted by the oyster industry in South Australia, with temperature regimes adapted to suit South Australian environmental conditions (Oysters South Australia, 2021). South Australia also has a response protocol to vibriosis notifications (Personal Communication Dowsett, 2022). If illnesses are reported, the protocol will be used for closure and re-opening of an affected harvest area. For closure, the number of illnesses reported by SA Health within one month of harvest of the associated products are considered; 1-5 illnesses associated with different harvest dates from the same harvest area will result in an investigation and ≥2 illnesses associated with the same harvest date or ≥5 illnesses with differing harvest dates from the same harvest area will result in a closure. For reopening of a closed harvest area, the *V. parahaemolyticus* level must be <3 MPN/g in 5 lots of 12 oysters tested in two consecutive weeks in a NATA accredited laboratory. Alternatively, pathogenic markers, *tdh* or *trh* measured by testing in a laboratory with PCR capability must be <10 per gram of shellfish meat. The South Australian protocol is based on the risk assessment and risk management procedure described in The National Shellfish Sanitation Programme (NSSP) guideline by the USA Food and Drug Administration (FDA, 2019).

5.5.3. International control measures

The Codex Alimentarius “Guidelines on the application of general principles of food hygiene to the control of pathogenic *Vibrio* species in seafood” describes some specific guidance on potential risk management strategies for the control of *Vibrio* in seafood (CODEX Alimentaris, 2010). According to this guideline, a risk evaluation associated with environmental factors and harvesting practices needs to be carried out factoring in the number of epidemiological cases and environmental parameters (air temperature, water temperature and salinity). Predictive models can be useful provided the model is validated using local data and can be used to identify factors that can be managed to reduce risk, such as those listed in the Tasmanian VCP and industry grower guide (Tasmanian Government, 2019a, Oysters Tasmania, 2019). Periodic monitoring for levels of *Vibrio* at various points of the distribution chain is recommended, and the involved personnel should be educated about temperature control and growth of *Vibrio* ((CODEX Alimentaris, 2010). A Good Hygiene Practices and Hazard Analysis Critical Control Points (HACCP) programme needs to be implemented to control *Vibrio* during post-harvest handling and processing (CODEX Alimentaris, 2010). The guideline recognises that general food hygiene controls, such as, temperature control and limiting cross contamination will control *Vibrio* in BMS (CODEX Alimentaris, 2010).

Many different control practices are implemented around the world. Examples are given in **Table 6**.

Table 6: International examples of food standards and control practices for *Vibrio*.

Country	Food Standard	Control practice	Reference
Australia	Not applicable	Cool chain requirements in the Australian Shellfish Quality Assurance Management Plan. *V. parahaemolyticus* management plans based on air and water temperature in some growing areas. Closures based on illness rates. FSANZ Microbiological compendium for ready to eat food: <3 CFU/g satisfactory, <3-100 CFU/g marginal, 100-10,000 CFU/g unsatisfactory, >10,000 CFU/g potentially hazardous.	**(**FSANZ, 2022)
New Zealand	Not applicable	*V. parahaemolyticus* management plans based on air and water temperature. Closures based on illness rates. FSANZ Microbiological compendium for ready to eat food also applies: <3 CFU/g satisfactory, <3-100 CFU/g marginal, 100-10,000 CFU/g unsatisfactory, >10,000 CFU/g potentially hazardous	(New Zealand Government, 2022, FSANZ, 2022)
USA	Not applicable	*V. parahaemolyticus* management plans based on air and water temperature. Closures based on illness rates. Some post-harvest processing practices approved. Tight supply chain temperature requirements.	(NSSP, 2019)
Canada	<100 *V. parahaemolyticus* per g shellfish in 5 samples. *V. cholerae* absent in 5 x 25 g samples or a pooled 125 g sample.	Water temperature monitoring and mandatory testing of shellfish for *V. parahaemolyticus* above certain triggers (temperature and illness). Tight temperature requirements at harvest and in the supply chain.	(Government of Canada, 2019, Government of Canada, 2020)
Japan	<100 *V. parahaemolyticus* per g fresh fish and shellfish for raw consumption and raw oysters. Not detected in boiled octopus, boiled crab	Potable water, tight temperature controls.	(Japan External Trade Organisation, 2011)
China	100 MPN/g acceptable limit, 1,000 MPN/g highest safety limit (contains sampling plan)		(National Health Commission of the People's Republic of China, 2021)
Hong Kong	Not applicable	Centre of Food Safety Guideline <20 CFU/g satisfactory, 20-10,000 CFU/g borderline, >10,000 CFU/g unsatisfactory	(Centre for Food Safety, 2014)
Singapore	100 CFU/g for ready to eat food		(Singapore Statutes Online, 2023)
Vietnam	No regulations		(USDA Foreign Agricultural Service, 2013)

5.5.4. Post-harvest procedures to reduce *Vibrio* risk

Post-harvest processing procedures can reduce the number of pathogenic *Vibrio* in BMS, however, these can impact the quality of the product, hence a balance is required between reducing the *Vibrio* numbers and retaining the acceptance of the product for consumers (FAO/WHO, 2020). The FAO/WHO report on “Advances in science and risk assessment tools for *V. parahaemolyticus* and *V. vulnificus* associated with seafood” has recommended several post-harvest processing technologies (FAO/WHO, 2021). These include cryogenic individual quick freezing with extended storage, high hydrolytic pressure, low dose gamma radiation, mild heat treatment and freezing (FAO/WHO, 2021). In addition novel treatments are being investigated such as submerging in acidic electrolyte water, use of citric acid, lactic acid, green tea extract and chlorine extract, antimicrobial photodynamic therapy, and biological control using predatory bacteria or bacteriophages (King et al., 2018a). Four post-harvest processing technologies namely cool pasteurisation, cryogenic individual quick freezing with extended storage, hydrostatic pressure processing and irradiation were approved by the US FDA to reduce *V. parahaemolyticus* to an acceptable level of <30 per gram (NSSP, 2019). Guidelines on validation of the pathogen reduction and verification of reduction during processing are available in the NSSP (2019).

5.5.5. Predictive models and forecasting for illness

Based on studies on growth of *V. parahaemolyticus* in Pacific Oysters, Tamplin et al. (2011) developed a predictive model for post-harvest *V. parahaemolyticus* growth (Fernandez-Piquer et al., 2011). The model was used to assess potential growth of *V. parahaemolyticus* and total viable counts in Pacific Oyster Tasmanian supply chain scenarios (Fernandez-Piquer et al., 2013). Both short (2.8 days from grower to consumer via one retailer) and long (6.2 days from grower to consumer including three wholesalers and one retailer) supply chains were considered in summer and winter. The predicted mean *V. parahaemolyticus* levels after consumer storage were similar for long and short supply chains in summer, although the percentage of oysters predicted to contain >1 CFU/g *V. parahaemolyticus* was higher for the short supply chains. In winter, predicted *V. parahaemolyticus* levels after consumer storage were lower for the long as opposed to short supply chain (Fernandez-Piquer et al., 2013).

Recently in Taiwan, a machine learning algorithm (Extreme Gradient Boosting) was employed to develop a predictive model for *V. parahaemolyticus* concentrations in oyster and seawater (Ndraha et al., 2021). This model demonstrated that SST, salinity, wind speed and pH of seawater strongly influenced the prediction of *V. parahaemolyticus* (Ndraha et al., 2021).

Various predicative models have been developed in the USA factoring in environmental parameters (Urquhart et al., 2015, Namadi and Deng, 2021, Davis et al., 2019). The model developed by Namadi and Deng (2021) was based on data collected from various growing areas in the USA, and it accurately predicted *V. parahaemolyticus* levels in oyster 1-4 days before harvest and identified SST, salinity and pH as the most influential factors for prediction. In 2005, the US FDA published a model for forecasting *V. parahaemolyticus* illnesses associated with raw oyster consumption, which made predictions for 24 regions/seasons based on geography, harvest methods and *tdh* positive strains (FDA, 2005a, FDA, 2005b). In 2011, FAO and WHO used some local data to adapt this model to predict *V. parahaemolyticus* illnesses associated with raw oysters in Australia, New Zealand, Japan and Canada (FDA, 2011). However, key factors from the original model were retained where local data could not be identified. For the Australian prediction they used US data for several factors including: the relationship between water temperature and *V. parahaemolyticus* levels; percentage of *tdh* or *trh*+ oysters; under-reporting factor; and consumption statistics. The publication acknowledged over-prediction of illness in Australia.

6. Evaluation of adverse health effects

6.1. Disease characteristics

Consumption of *V. parahaemolyticus* contaminated raw or undercooked seafood may result in acute inflammatory gastroenteritis, characterised by watery diarrhoea, abdominal cramps, nausea, vomiting, fever and chills (Barker and Gangarosa, 1974, Hlady and Klontz, 1996). In most cases the infection is self-limiting and of short duration, however there may be a need for hospitalisation. On rare occasions, septicaemia may develop resulting in fever, hypotension (dangerously low blood pressure) and swelling of extremities, and/or haemorrhagic bullae (blistering skin lesions). The incubation period ranges from 12 to 96 hours, and symptoms can last from 2 hours to 10 days (median of 3 days) (Barker and Gangarosa, 1974).

Vibrio vulnificus also causes inflammatory gastroenteritis which may progress to septicaemia, fever, hypotension and haemorrhagic bullae. Whilst *V. vulnificus* infections are not as common as *V. parahaemolyticus* (Newton et al., 2012), progression to lethal septicaemia is more common with *V. vulnificus*, resulting in *V. vulnificus* being responsible for the majority of reported seafood-related deaths in the US (Jones and Oliver, 2009).

Exposure of open wounds to either *V. parahaemolyticus* and *V. vulnificus* can result in serious infections that may progress to cellulitis, ecchymoses and bullae; fatalities rates are lower than those for systemic septicaemia (Jones and Oliver, 2009).

Vibrio parahaemolyticus may cause outbreaks in association with food consumption but more commonly causes sporadic cases of illness (WHO/FAO, 2011). In contrast, gastroenteritis outbreaks caused by *V. vulnificus* are yet to be reported (FAO/WHO, 2005).

Much of our understanding of the public health implications of *Vibrio* infections comes from the USA where vibriosis has been a nationally notifiable disease since 2007 (Newton et al., 2012). Data from the USA surveillance programs COVIS[2] and FoodNet were used to model nationwide illnesses (Scallan et al., 2011), resulting in an estimate of annual *V. parahaemolyticus* illness burden from consumption of seafood of 34,664 cases (90% credible interval of 18,260 - 58,027), with a hospitalisation rate of 22.5% and a death rate of 0.9%. In contrast, the illness rate for *V. vulnificus* was estimated to be much lower at 96 cases annually (90% credible interval of 60 - 139), but with higher hospitalisation and death rates of 91.3% and 34.8%, respectively. These modelled estimates are similar to actual rates of illness, hospitalisation and death reported in Newton et al. (2012) for *V. parahaemolyticus* but lower than *V. vulnificus,* noting that the latter report includes wound infections.

Foodborne vibriosis is acknowledged to be increasing worldwide (Baker-Austin et al., 2017) and current estimates of USA annual *Vibrio* illnesses from the Centre for Disease Control and Prevention are 80,000 cases per annum, 52,000 of which are associated with food, and 45,000 of which are caused by *V. parahaemolyticus* (CDC, 2019).

Patients with underlying medical conditions (particularly liver disease or alcoholism, but also diseases such as cancer, renal disease, and diabetes) are more likely to develop septicaemia from *Vibrio* infections (Liao et al., 2015, Hlady and Klontz, 1996).

Other non-cholera *Vibrio* spp. reported to cause illness in the USA include *Vibrio alginolyticus* (third most common reported *Vibrio* spp. causing infection with similar hospitalisation and death rates to *V.*

[2] Cholera and Other *Vibrio* Illness Surveillance

parahaemolyticus), *Vibrio cholerae* non-O1 and non-O139, *V. fluvialis, V. mimicus, V. hollisae, Vibrio damsela* and *V. metchnikovii* (Newton et al., 2012, Hlady and Klontz, 1996).

6.2. *Vibrio parahaemolyticus* dose-response

Limited dose-response studies for vibriosis caused by *V. parahaemolyticus* exist (WHO/FAO, 2011, USFDA, 2005). The USA Food and Drug Administration's risk assessment conducted in 2005 created a dose-response model based on limited data from three human clinical feeding studies conducted between 1958 and 1974 using TDH positive *V. parahaemolyticus* strains (USFDA, 2005). The risk assessment assumed that all strains were equally virulent, however outbreak data collected since the risk assessment indicates this is not the case (King et al., 2018b). The dose-response modelled was adapted to better align with illness rates found in the USA, adjusting for the fact that the clinical studies fed the pathogen without a food matrix and administered antacids to reduce stomach acidity.

The dose-response model developed showed that illness can occur after low doses of *V. parahaemolyticus* (<0.001% probability of illness from consumption of 10,000 *V. parahaemolyticus* cells, which translates to 50 cells/g from a 200 g meal). The probability of illness increased as the dose increased, such that there was a 50% chance of illness following a dose of 10^8 cells (5 x 10^5 cells/g in a 200g meal). The risk assessment found a greater probability of development of septicaemia in patients with chronic medical conditions (five out of every 7 cases of septacaemia were immunocompromised) (USFDA, 2005).

A *V. parahaemolyticus* risk assessment was conducted by the WHO/FAO in 2011 (WHO/FAO, 2011) using this dose-response model and again assuming that all strains were equally virulent. It should be noted that the number of illnesses estimated from Australian oysters produced by the WHO/FAO risk assessment was significantly higher than illnesses recorded in Australia (1,700 predicted illnesses per annum from Wallis Lakes, New South Wales over 18 years compared to 2 cases recorded).

6.3. *Vibrio vulnificus* dose response

Human dose-response trials have not been conducted for *V. vulnificus*. A dose-response curve for the Gulf of Mexico was modelled in the FAO/WHO (2005) *V. vulnificus* risk assessment based on an estimate of *V. vulnificus* consumption through locally produced shellfish and the recorded illnesses. The month and year specific dose exposure per serving was estimated from:

- relationships between *V. vulnificus* numbers in oysters at harvest and surface water temperature;
- measured temperatures from the Gulf Coast water;
- post-harvest handling assumptions and adjustment of *V. vulnificus* numbers in oysters according to growth and survival models;
- the monthly average number of servings consumed per month by the susceptible population, assuming 50% landed catch was consumed raw, 7% of these were consumed by susceptible people and 197 g serves.

The dose exposure was then compared to the number of oyster-associated cases of *V. vulnificus* recorded by the CDC in each month of each year from 1995 to 2001. A Beta-Poisson regression approach was then used to produce a dose-response curve, and the uncertainty analysis conducted by considering alternative datasets within the expected variability of the number of cases reported and the potential *V. vulnificus* numbers at harvest, but assuming the same harvest-to-consumption data as listed above. The developed dose-response model showed higher doses of *V. vulnificus* were necessary to cause illness (0.001% probability of illness from consumption of approximately 240,000 *V. vulnificus* cells, which translates to 1,200 cells/g from a 200 g meal). The probability of illness increased slowly as

the dose increased, such that there was 0.003% chance of illness following a dose of approximately 10^7 cells (50,000 cells/g in a 200g meal).

It should be noted that the dose-response model calculated is specific to the Gulf of Mexico as the relationship between *V. vulnificus* levels at harvest and surface water temperature is likely to vary with regions, environmental conditions such as salinity, handling practices, and species of shellfish. Furthermore, consumption patterns and the proportion of susceptible individuals may also be different in other regions.

6.4. Surveillance systems in Australia

To understand the historic illness statistics for vibriosis we need to understand the Australian surveillance and reporting systems, and individual responses to gastroenteritis infections. Australia's National Notifiable Diseases Surveillance System (NNDSS) began in 1991 (Gibney et al., 2017). Diseases prior to this period are summarised in the notifiable diseases 1917 – 1991 database and publication (Hall, 1993b, Hall, 1993a). Cholera (toxigenic *V. cholerae* O1 or O139) has been a nationally notifiable disease since the NNDSS inception (Gibney et al., 2017). However, the notifiable status of other *Vibrio* infections is variable across the states, as listed below. **Table 7** highlights that *V. parahaemolyticus* is notifiable in Northern Territory, Tasmania, South Australia and Western Australia, but *V. vulnificus* is only notifiable in Northern Territory and Tasmania. Regardless of notifiable disease status, outbreaks[3] of vibriosis will be reported in every state if they are detected.

Table 7: Notifiable status of *Vibrio* infections in Australia.

State	Notifiable disease status (year introduced, data provided by OzfoodNet representatives in each state)	*V. parahaemolyticus* reported in Notifiable disease database 1917-1991
Northern Territory	All *Vibrio* infections (unknown but prior to 2012)	1985-1991
Queensland	Not notifiable	
New South Wales	Not notifiable	1984-1991
Australian Capital Territory	Not notifiable	
Victoria	Not notifiable	
Tasmania	All *Vibrio* infections (since 1990's)	1989-1991
South Australia	*V. parahaemolyticus* (2016)	1982-1991
Western Australia	*V. parahaemolyticus* (1985)	1984-1991

Under-reporting of illness is a key factor to be considered. Many individuals with gastroenteritis may not present to the medical system, or if they do present, the health provider may not consider it necessary to investigate the cause. If the cause is investigated, pathology methods vary both across and within states and may not target identification of *Vibrio* spp., particularly in states where vibriosis is a not a notifiable disease. The Center for Disease Control in America estimates only one in 20 cases of *V. parahaemolyticus* are reported to public health authorities (WHO/FAO, 2011).

[3] Two or more illnesses within a short time frame resulting from ingestion of a common food

6.5. Epidemiology/Illnesses in Tasmania

There were 27 vibriosis cases associated with Tasmanian seafood reported to the Tasmanian Department of Health (DoH) between January 2016 and June 2022 inclusive (with these including case notifications from within Tasmania and reports from interstate jurisdictions were investigations identified product was sourced from Tasmania). The majority of cases were sporadic, with only one outbreak identified. Twenty-six of these cases were associated with *V. parahaemolyticus* in oysters (**Table 8**) and one was *Vibrio cholera* non O1/139 possibly associated with flathead (this is not included in **Table 8**). The average time from consumption to illness was 1 +/- 1 day and *V. parahaemolyticus* genotypes included MLST 36 and 50.

Table 8: Vibriosis associated with oysters harvested from Tasmanian growing areas between 2016 and 2022, inclusive. DoH data (2022).

Region	No. illnesses (no. hospitalisation)	Date	Commercial/ recreational	Sporadic/ outbreak	MLST	Seafood
North West	1	Jan-2019	Commercial	Sporadic	36	Oysters
Moulting Bay	11 (4)	Jan-2016	Commercial	Outbreak		Oysters
Moulting Bay	1	Feb-2019	Commercial	Sporadic		Oysters
Moulting Bay	1	Feb-2019	Commercial	Sporadic		Oysters & seafood platter
Mid East	1	Jan-2019	Commercial	Sporadic		Oysters
Wedge Island	1	Apr-2022	Recreational	Sporadic	50	Oysters & scallops
South East	1	Mar-2022	Recreational	Sporadic		Oysters
South East	1	Mar-2022	Recreational	Sporadic	50	Oysters
Bruny Island	1	Apr-2017	Both	Sporadic		Oysters
Bruny Island	1	Apr-2022	Commercial	Sporadic	50	Oysters
Huon Estuary	1	Jan-2022	Recreational	Sporadic	36	Oysters
Unknown source	5	2017, 2019, 2021	Commercial	Sporadic		Oysters

The first *V. parahaemolyticus* illnesses were recorded at Moulting Bay during a marine heatwave in summer 2015/2016. Multiple illnesses also occurred in summer 2019 across a variety of sites, and in the summer of 2022 in the south-east of the state.

Air temperatures in Tasmania tended to be warmer than average between 2015 and 2022 (Bureau of Meteorology: BOM) data summarized in**Table 9**; (BOM, 2023). However, elevated temperature has not always resulted in illness, as above average temperatures occurred without known illness in 2016/2017 and 2019/2020.

Table 9: Summary of average summer seasonal temperatures and rainfall in Tasmania and corresponding illnesses by region.

Summer	BOM temperature comments	BOM rainfall comments (east Tasmania)	Illness (Region)
2015/2016	Record warm air temp. Marine heat wave	Below average	Outbreak (11 in NE)
2016/2017	Average	Dry	Sporadic (1 in SE, 1 unknown)
2017/2018	Above average	Wet	
2018/2019	Very warm	Mostly dry	Sporadic (4 in NW to SE, 1 unknown)
2019/2020	Record hot days in December and Jan	Below average	
2020/2021	Cooler than average in Dec and Feb, warmer in Jan	Wetter in NE, average elsewhere	Sporadic (1 unknown)
2021/2022	Above average	Record dry	Sporadic (5 sites, SE & Bruny)

6.6. Epidemiology/Illnesses in Australia

Published data on vibriosis in Australia are rare. Information on cases can be found in four main data sources: Notifiable diseases database 1917-1991 (Hall, 1993b), New South Wales surveillance (Kraa, 1995), Harlock et al. (2022) and a national risk project (Food Science Australia and Minter Ellison Consulting, 2002).

Between 1984 and 1995, 40 cases of sporadic illness (no outbreaks, no deaths, two hospitalisations) of *V. parahaemolyticus* associated with shellfish or unknown food sources were reported to Australian public health authorities (Kraa, 1995, Hall, 1993b, Food Science Australia and Minter Ellison Consulting, 2002); none of these were from Tasmania, although vibriosis was not a notifiable disease in Tasmania for much of this period. One large outbreak (>148 illnesses) associated with New South Wales shellfish occurred in 1990, with both *V. parahaemolyticus* and elevated *Escherichia coli* levels detected in shellfish. Several large outbreaks (177 illnesses, one death) associated with imported prawns also occurred during this period. The combined *Vibrio* infection rates per state ranged from between 0.02 and 0.17 per 100,000, adjusted for areas where the disease was not notifiable (Hall, 1993a).

Since 2002 there have been 6 outbreaks (>300 cases) in Australia and 29 sporadic illnesses (no deaths) of *V. parahaemolyticus* reported from states where the disease is notifiable (Harlock et al., 2022, www.sahealth.gov.au). When oysters where the confirmed vector of an outbreak, they were identified as coming from either Tasmania (2016) or South Australia (one outbreak in 2016 and two in 2021). Generally, case numbers associated with these outbreaks have been low, although the two outbreaks that began in 2021 recorded 21 and over 290 illnesses. Sporadic illnesses of *V. parahaemolyticus* appear more frequent, with 29 cases reported from states where the disease is notifiable between 2005 and 2019 inclusive (Harlock et al., 2022).

Combined outbreak and sporadic illnesses reported in states with *V. parahaemolyticus* as a notifiable disease (Harlock et al., 2022) are provided in **Table 10**. The average infection rates for the period of reporting are similar to the range of rates for each state published in (Hall, 1993a), based on data from 1984 to 1991 (noting the latter included a large number of illnesses from prawns).

Table 10: Calculated *V. parahaemolyticus* infection rates for states where it is notifiable, based on data in Harlock et al. 2022.

State	Notifiable period covered by data (years)	Number of *V. parahaemolyticus* foodborne illnesses reported during notifiable period[1]	Sum yearly population during notifiable period[2]	*V. parahaemolyticus* infection rate per 100,000, range per year (average over all years)
Northern Territory	2005 – 2019 (15)	2	3,487,300	0 - 0.81 (0.06)
Western Australia	2005 – 2019 (15)	30	35,542,473	0 - 0.59 (0.08)
South Australia	2016- 2019 (4)	9	6,925,974	0.06 - 2.3 (0.13)
Tasmania	2005 – 2019 (15)	20	7,647,507	0 - 2.70 (0.30)

[1] (Harlock et al., 2022)
[2] (ABS, 2023)

Illness from *V. vulnificus* is rarely reported in Australia. The National Risk Validation Project 2001 (Food Science Australia and Minter Ellison Consulting, 2002, reported in Sumner, 2011) reviewed historic data and found 6 cases of *V. vulnificus* infection related to oysters between 1988 and 1990 inclusive, 3 of which resulted in death.

6.7. Illnesses in overseas

Currently no global surveillance framework exists for *Vibrio* spp. and few countries have national surveillance systems. *Vibrio parahaemolyticus* reports began in Japan in the 1960s, where vibriosis remains a significant health issue with 500-800 outbreaks reported annually, affecting around 10,000 people (WHO/FAO, 2011). In the 1970s *V. parahaemolyticus* illness was recorded from locations in the Atlantic, Pacific and Gulf states of USA, followed by Europe, Africa, New Zealand and most Asian countries (Baker-Austin et al., 2018). A diverse group of strains were responsible for the illnesses. In 1996 a genetically distinct variant of the strain O3:K6 emerged in India that rapidly spread throughout south-east Asia within one year (Nair et al., 2007b). By 2007 this pandemic strain was responsible for illness in Bangladesh, Chile, France, Japan, Korea, Laos, Mozambique, Peru, Russia, Spain, Taiwan, Thailand, and the USA. In 2012 another pandemic strain ST36 evolved from the Pacific northwest that showed high virulence and spread to the western seaboard and Spain (Baker-Austin et al., 2018). The Centre for Disease Control and Prevention currently estimate annual illnesses from *V. parahaemolyticus* in the USA to be around 45,000 (CDC, 2019), most of which occur as sporadic illnesses or small local outbreaks. This translates to an infection rate of 0.136 per 100,000 (CDC, 2022).

Surveillance data for *V. vulnificus* is sparse as many countries do not collect this data in a systemised manner (FAO/WHO, 2005). Illness from *V. vulnificus* is rare with virtually all cases occurring in people with an underlying medical condition. Thus, illness reports are sporadic and outbreaks do not occur.

7. Evaluation of risks

7.1. Uncertainty

There are numerous knowledge gaps affecting the confidence of assessing risk from vibrios in Tasmanian commercial bivalve shellfish production.

The confidence in illness statistics is unknown, however illnesses are likely to be underreported due to: the short-term and relatively inconsequential nature of most illnesses and the shortfall in regional doctors resulting in many patients not presenting to a doctor; illnesses may not be investigated even if patients do present to a doctor; pathology laboratories may not have the required test suite to analyse for *vibrios*; illnesses may not be notified to health authorities, depending on the notifiable status of vibriosis the states/territory where the illness occurs.

Furthermore, the size of the highly susceptible population in Australia is unknown. Most shellfish (97%) are sold domestically, and Tasmanian product may enter any state or Territory. There are a wide range of immunocompromising chronic diseases that increase susceptibility to vibriosis, making it hard to predict the proportion of the population that is more susceptible both to illness in general and those likely to experience more severe symptoms.

The bacterial dose required to cause illness is particularly uncertain and is likely to vary between genotypes of pathogenic *Vibrio*. This impacts the assessment of whether those doses exist in Tasmanian shellfish, which may also be influenced by the level of sensitivity of the test methods used and the presence of VBNC *Vibrio*. The withdrawn quantitative AS 5013.18 2010 for *V. parahaemolyticus* had a limit of detection of <3 MPN/g, while the new qualitative (presence or absence) AS 5013.18.1 has a limit of detection of <10 CFU/g (not detected in 0.1 g) or <100 CFU/g (not detected in 0.01g). The new qualitative standard has provision for PCR confirmation of *tdh*+ and *trh*+ *V. parahaemolyticus* strains but this is not a requirement of the testing laboratory if biochemical confirmation of *Vibrio* strains is adopted in preference to PCR. Methods using non-selective pre-enrichment, as opposed to direct plating onto selective media, increase chances of resuscitating VBNC *Vibrio* (FAO/WHO, 2016).

The survey reported in this document has determined the prevalence and levels of *V. parahaemolyticus* and *V. vulnificus* in Tasmanian growing areas over 2.5 years, between January 2020 and April 2022. Climate analysis by the Bureau of Meteorology shows that 2020 and 2022 were wetter than average years, and 2021 and 2022 were warmer than average years (BOM, 2023). Prevalence and levels of *Vibrio* may change significantly, particularly during marine heat wave events. One such marine heat wave was recorded in Tasmania in the summer of 2015/2016 (Oliver et al. 2017), which coincided with the timing of the 2016 Moulting Bay vibriosis outbreak. Very warm events were also observed in the summer of 2018/2019 and coincided with several sporadic Tasmanian shellfish vibriosis cases. Water temperature was a significant primary or secondary predictor of *V. parahaemolyticus* risk in most of our surveyed harvest areas (**Table 2**)

The range of pathogenic *Vibrio* found in Tasmania in unknown, although both ST 36 and ST 50 have been identified in clinical cases associated with Tasmanian shellfish consumption. These strain types were also isolated from clinical samples associated with the 2021 South Australian oyster outbreak (Government of South Australia, 2021, Government of South Australia, 2022). Although strains containing the pathogenicity determinants *tdh* and/or *trh* were detected during the Tasmanian survey and with a higher prevalence in regions with previous implicated illness (**Figure 3**), no pure bacterial cultures containing these specific genes were isolated using microbial culture. This may be an artifact of the selective culture-based isolation technique used or a combination of the limited culture based isolation effort undertaken during the survey, in conjunction with inherent lower prevalence of these *tdh*+ and *trh*+ *V. parahaemolyticus* strains compared to total *V. parahaemolyticus*. Tasmanian *V. parahaemolyticus* isolates were collected and typed using WGS MSLT indicating the presence of a range of environmental ST types, some of which were unique to Tasmania. Some of our ST types have previously been identified as environmental strains containing single or multiple antibiotic resistance. No ST types implicated in sporadic or outbreak shellfish-related Australian vibriosis were identified in our limited culture collection, although three ST types (ST12, ST141 and ST396) have been isolated from

clinical cases elsewhere. The ST types of our *tdh*+ and *trh*+ *V. parahaemolyticus* detections are unknown because the strains could not be isolated.

The variation in distribution of *Vibrio* contaminated shellfish across leases and the possibility of the occurrence of individual shellfish with high concentrations of *Vibrio* is unknown. Thus, sampling plans may not adequately address variability in the growing area, affecting prevalence and quantity estimates. The variation in accumulation of *Vibrio* in shellfish of different ploidy levels in also unknown, affecting prevalence and quantity estimates. Ploidy has been shown not to influence *Vibrio* spp. levels (*V. parahaemolyticus* and *V. vulnificus*) in Eastern Oysters, although ploidy has been shown to influence *Vibrio* spp. levels (host pathogenic species) in Pacific Oysters, with a positive association of *Vibrio* infection and gonodal tissue (Grodenska et al., 2019, De Decker et al., 2011).

The proportion of shellfish harvested from each region during summer months when *Vibrio* levels are likely to be higher is unknown but is likely to be significant since Christmas and Easter represent periods of major demand. However, lower volumes may also be harvested since diploid oysters spawn between December and February and are out of condition for two to three months after spawning. Whilst Tasmanian farmers implement post-harvest temperature controls in accordance with state and ASQAAC requirements, no market surveys or supply chain studies are reported so the effectiveness of these in controlling *Vibrio* growth is unvalidated. Modelling of Tasmanian supply chains has however indicated that the most important factor affecting *Vibrio* growth post-harvest is their numbers/levels at the point of harvest (Fernandez-Piquer et al., 2013).

The supply chain length associated with each harvest region is unknown, so we have assumed the same supply chain lengths (4 days) across all harvest areas. We also do not have any information on consumer storage of shellfish post retail or farm-gate purchase, so we have not considered these in our analysis. We have used the Oyster Refrigeration Index tool to estimate the growth of *V. parahaemolyticus* post-harvest under various scenarios fitting the time into the cool chain requirement according to ASQAP and the Tasmanian VCP guidelines (Fernandez-Piquer et al., 2011, Fernandez-Piquer et al., 2013, ASQAAC, 2022, Tasmanian Government, 2019a). The accuracy of the Oyster Refrigeration Index in estimating *V. parahaemolyticus* growth post-harvest, and while being maintained in the cool chain is unknown. We noticed that *V. parahaemolyticus* prediction levels often dropped significantly (by up to 50%) between levels reached following entry into the cool chain (10^{o}C) and levels following maintenance within the cool chain (8^{o}C) for up to 4 days. The Oyster Refrigeration Index model has been used to estimate growth *of V. parahaemolyticus* in naturally contaminated Pacific Oysters from New Zealand and found to vary significantly from measured results. This led to the development of a new model of growth from Pacific Oysters naturally contaminated with *V. parahaemolyticus* from New Zealand growing areas (Cruz et al., 2022). Fitted growth rates of *V. parahaemolyticus* in the New Zealand study were considerably lower than previously reported using the Oyster Refrigeration Index, and the hypothetical minimum growth temperature was also lower in the New Zealand model (8^{o}C versus 13^{o}C (Cruz et al., 2022). Fernandez-Piquer et al (2011) showed *V. parahaemolyticus* declines in artificially contaminated Pacific Oysters at temperatures from 12.6 to 3.6^{o}C, while Cruz et al (2022) showed that the average growth rate of *V. parahaemolyticus* in naturally contaminated Pacific Oysters at 10^{o}C was close to zero.

7.2. Previous risk assessments for *Vibrio* in Australian bivalve shellfish

There has been no previous risk assessment specific to *Vibrio* in Tasmanian oysters. In 2003 Lewis et al. published data on pathogenic *V. parahaemolyticus* in Australian oysters to support an FAO/WHO global risk assessment. The data included prevalence and level of *V. parahaemolyticus* measured in a limited number of oyster samples sourced from New South Wales, Tasmania and South Australia (n=

20, 10 and 10, respectively) taken at one point in time. The FAO risk assessment (FAO/WHO, 2011) estimates 91 annual illnesses of vibriosis associated with oysters per annum in Australia. This was acknowledged to be an over-estimate compared to illnesses being reported and USA determined rate of under reporting.

7.3. Evaluation of risk from *Vibrio* in commercially produced Tasmanian bivalve shellfish

7.3.1. *Vibrio vulnificus* risk

Vibrio vulnificus was detected during the survey, but only during the summer/autumn periods and generally at low levels (<1 MPN/g). A higher prevalence was observed in the north and north-east and higher state-wide prevalence and levels were observed in the summer/autumn of 2021/22. *V. vulnificus* was not detected in Duck Bay, Boomer Bay, Great Bay or Fleurtys Point. In other areas it was often not detected in all three summer/autumn sampling periods.

Only shellfish (oysters and mussels) from Great Swanport had consistent detections of *V. vulnificus* in all three summer/autumn periods with levels of 35-460 MPN/g detected in the 2021/22 summer/ autumn period. The drivers for *V. vulnificus* in Great Swanport were maximum river flow of the Apsley River and 1 day rainfall prior to sampling. Temperature was not a significant driver of *V. vulnificus* at harvest in Great Swanport in the summer/autumn months although it was not detected in winter/spring.
There is no evidence that *V. vulnificus* presents a substantial risk for the Tasmanian oyster industry. There has been no illness reported in Tasmania. Given the severity of the disease, illness is likely to have been reported if it occurs. Of all the growing areas, Great Swanport had the highest prevalence and numbers of *V. vulnificus* and, therefore, presents the greatest risk.

7.3.2. Evidence of shellfish related *V. parahaemolyticus* vibriosis and severity of illness

All regions, although not all harvest areas, surveyed in Tasmania have had illness reported since 2016. All illnesses have been reported during the summer/autumn (January to March), associated with warmer weather and when there is a greater prevalence and level of *V. parahaemolyticus* at production. Most illnesses have been sporadic with only one outbreak reported in 2016, at a time when a marine heat wave was impacting Tasmania. Regardless of whether illnesses have been sporadic or an outbreak, the number of illnesses associated with Tasmanian shellfish consumption have been low. Some of these were also reported from shellfish not associated with commercial harvest.

For most individuals suffering foodborne vibriosis (*V. parahaemolyticus*) the severity of illness will be low and short-lived. However, the immunocompromised may suffer severe illness. In the 2016 outbreak 4 of the 11 reported cases were hospitalised.

7.3.3. Occurrence of *V. parahaemolyticus* in shellfish at harvest

The maximum average summer daily air temperatures, in the growing areas surveyed in Tasmania, were in the range of 19°C to 23.5°C. The summer three-day average water temperatures ranged from 16.1-17.6°C, while the minimum and maximum summer three-day averages ranged from 10.3-16.1°C and 16.8-22.8°C, respectively. For most growing areas, the minimum water temperature linked with *V. parahaemolyticus* growth (**Table 2**) was close to or below the minimum three-day average recorded in the growing area **Table 11**, indicating the likelihood of detecting *V. parahaemolyticus* across the whole summer season. The exceptions to this were Little Swanport and the Bruny region, where the minimum three-day average water temperatures were more than a degree below the minimum temperature associated with *V. parahaemolyticus* growth.

The prevalence and levels of *V. parahaemolyticus* in Tasmanian commercial growing areas during this survey was variable but tended to be higher in the north and north-east of the state, and decreased progressively further south, as summarised in **Table 11**.

Significant numbers of *V. parahaemolyticus* (>100 MPN/g) were only found in summer/autumn (defined as November to end of April) and again, mainly associated with the north and north-east regions (**Table 11**). Environmental predictors for increased *V. parahaemolyticus* numbers varied between growing areas, but temperature was the predominant predictor in all areas, either as a primary driver or in combination with another environmental driver (**Table 2**).

Most clinical isolates of *V. parahaemolyticus* are *tdh*+, *trh*+, or carry both genes (Bhoopong et al., 2007). Clinical strains of *V. parahaemolyticus* lacking *tdh* and *trh* have been isolated from 4-10.9% of specimens (Banerjee et al., 2014, Bhoopong et al., 2007). These strains may carry other unknown virulence mechanisms although heterogeneous populations of *V. parahaemolyticus* have been isolated from a single patient highlighting the importance of not basing epidemiological characterisation of *V. parahaemolyticus* on a single colony isolation from the patient (Bhoopong et al., 2007).

Internationally, environmental *V. parahaemolyticus* isolates have had a low reported prevalence (1-2%) of *tdh*+ and *trh*+ genes (Bhoopong et al., 2007). In our survey *V. parahaemolyticus* strains containing the pathogenicity *tdh* and *trh* determinants were only detected in the summer/autumn months with a prevalence of 0-21% and 0-18%, respectively, depending on growing region. The prevalence of *V. parahaemolyticus* containing both pathogenicity determinants ranged from 0-7%. The state-wide prevalence of *tdh*+, *trh*+ and t*dh*+/*trh*+ *V. parahaemolyticus* detected during the summer/autumn survey period was 6.5%, 5.9% and 2.3%, respectively. Previous Tasmanian *V. parahaemolyticus* studies have either not investigated the presence of *tdh*+/*trh*+ *V. parahaemolyticus* strains or reported prevalence ranging from 0-20% for *tdh*+ and 0-17% for *trh*+ *V. parahaemolyticus* (Lewis et al., 2003, Madigan et al., 2017).

7.3.4. Growth of *V. parahaemolyticus* post-harvest and effectiveness of control measures

All shellfish production areas in Tasmania need to adhere to ASQAAC guidance on temperature control post-harvest, specifically that shellfish harvested for human consumption must be reduced to and kept at ≤10°C within 24 hr of harvest (ASQAAC, 2022). Tasmanian shellfish harvested from growing areas which have been implicated in foodborne vibriosis are required to implement a VPC which stipulates more stringent control measures for product moving into the cool chain (Tasmanian Government, 2019a).These stringent controls are that from November 1st – April 30th, the time from harvest to cool chain (≤10°C) must not be greater than 12 hours. Furthermore, when the ambient air temperature is >30°C or when the water temperature at the depth where oysters are harvested is >19°C, the time from harvest to cool chain must not be greater than 7 hours. The three-day average water temperature in all growing areas surveyed except for Great Oyster Bay and Great Bay exceeded 19°C at some point during summer/autumn during the survey. Currently there are only four harvest areas mandated to implement a VCP plan, although it is highly recommended for other harvest areas (Tasmanian Government, 2019a).

The Tasmanian oyster supply chain is complex and varied. The majority of product is sold interstate with short to long (3-6 days) supply chains, depending on destination. In the current evaluation we have assumed all Tasmanian growing areas have the same supply chain lengths. The failure of temperature control at any point(s) in the supply chain can significantly increase vibriosis risk, especially in summer/autumn when total *V. parahaemolyticus* and pathogenic strain prevalence and levels are higher. Growing regions in the north and north-east appear to have inherently higher levels of *V. parahaemolyticus* at production. Most oysters are harvested from March to November with lower

volumes harvested from December to February when diploid oysters spawn. Harvest peaks are observed around Easter and Christmas due to consumer demand. If growing areas farm triploids they are able to sell more product during the higher risk warmer months which may increase the risk of illness.

To assess the growth of *V. parahaemolyticus* post-harvest, we used the Oyster Refrigeration Index online tool, in conjunction with the ASQAAC and the Tasmanian VCP guidance on time for product into the cool chain. We have assumed a very efficient cool chain that rapidly (within 2 hrs) brings the shellfish down to the target temperature. However, realistically, it may take much longer to achieve this, dependent on:

- The power of the cooling unit (truck-based refrigeration units are likely to be less powerful than those in a shellfish processing facility).
- The set operating temperature (it will typically take long time for the temperature of the product to drop the final few degrees.
- The volume of shellfish placed in the cooler. The more heat load, the longer it will take to reach the target temperatures.
- How the shellfish are laid out in the chiller. A large mass with limited airflow through it will be slower to cool than if shellfish are in layers with good airflow between them.

Although a super-efficient cool chain was assumed in our assessment (**Table 12**), a worst-case scenario of oysters held at ambient temperatures post-harvest prior to entry into the cold chain under ASQAAC (22 hrs) and the two levels of VPC (10 hrs or 5 hrs) was also assumed.

The minimum and maximum of our observed summer/autumn water temperatures (maximum three-day average) and assumption of ambient air temperatures of 15ºC, 20ºC, 25ºC and 30ºC were used as inputs into the model. Initial post-harvest contamination levels of *V. parahaemolyticus* was assumed at 10 cells/g, 100 cells/g and 1,000 cells/g based on levels observed during our survey and FSANZ guidelines in ready-to-eat food. The assumptions used in post-harvest *V. parahaemolyticus* modeling are shown in **Table 12**.

The results of the post-harvest *V. parahaemolyticus* growth are shown in **Table 13**, When interpreting these results tt should be noted that the Oyster Refrigeration Index was determined using *V. parahaemolyticus* strains isolated from shrimp in Thailand and injected into Pacific Oysters and may not be representative of all *V. parahaemolyticus* growth in this species (Fernandez-Piquer, 2011). Recent work by Cruz et al. (2022) found lower growth rates in Pacific Oysters naturally containing with *V. parahaemolyticus* from New Zealand. This work suggests that the Oyster Refrigeration Index may overestimate growth above 15ºC, and overestimate decline during refrigeration. None-the-less, **Table 13** shows that exposure to ambient air temperature and the duration of this exposure, along with initial *V. parahaemolyticus* contamination levels, had the greatest impact on risk of *V. parahaemolyticus* reaching unacceptable levels.

Table 11: Summary of Tasmanian *V. parahaemolyticus* (Vp) survey results.

Region	Growing areas within region	Summer 3-d Av water temp. range and (Av water temp ± SD) ºC	Summer average max daily air temp ± SD ºC	Summer Vp prevalence range (max Vp level)	Summer *tdh*+, *trh*+ or (*tdh*+/*trh*+) Vp prevalence %	Winter Vp prevalence range (max Vp level)	Intertidal versus subtidal production	Coastal versus estuarine production
North West	Duck Bay	12.7 - 19.8 (16.1 ± 2.3)	20.4 ± 3.1	91-100% (460 MPN/g)	14, 17 (7)	0-33% (1.4 MPN/g)	Inter-tidal	Estuarine
Moulting Bay	Moulting Bay	14.7 - 23.3 (17.6 ± 2.2)	20.6 ± 3.3	67-89% (110 MPN/g)	21, 7 (3)	17-20% (0.36 MPN/g)	Inter & sub-tidal	Estuarine
Upper East Coast	Great Oyster Bay[1]	15.6 - 16.9 (16.5 ± 0.6)	23.5 ± 4.3	60% (0.3 MPN/g)	0	0% (ND[2])	Sub-tidal	Coastal
	Great Swanport	12.1 - 20.3 (17.3 ± 2.2)	19.4 ± 2.8	88-100% (1100 MPN/g)[3] 78-100% (460 MPN/g)[4]	5,18 (5)[3] 5, 14 (0)[4]	0-17% (0.36 MPN/g)[3] 0% (ND)[4]	Inter & sub-tidal	Estuarine
	Little Swanport	11.3 - 20.7 (16.4 ± 2.0)	19.0 ± 2.8	73-100% (460 MPN/g)	3, 3 (0)	17-33% (0.72 MPN/g)	Inter & sub-tidal	Estuarine
Mid East Coast	Boomer Bay	12.2 - 19.6 (16.3 ± 2.0)	19.1 ± 5.0	33-75% (23 MPN/g)	7, 3 (3)	0% (ND)	Inter & sub-tidal	Estuarine
	Boomer Bay East	13.1 - 21.8 (16.9 ± 2.2)	21.0 ± 3.6	43-88% (9.2 MPN/g)	4, 0 (0)	0-20% (0.36 MPN/g)	Inter & sub-tidal	Estuarine
South East Region	Pipe Clay Lagoon	11.3 - 21.1 (16.2 ± 2.6)	22.1 ± 5.9	25-63% (3.6 MPN/g)	3, 3 (3)	0% (ND)	Inter-tidal	Estuarine
	Pitt Water	12.4 - 22.0 (16.8 ± 2.7)	21.8 ± 4.4	13-60% (16 MPN/g)	0, 0 (0)	0% (ND)	Inter-tidal	Estuarine
Bruny Region	Great Bay	13.2 - 19.2 (16.5 ± 1.8)	20.1 ± 3.8	8-50% (11 MPN/g)	3, 0 (0)	0% (ND)	Sub-tidal	Estuarine
	Fleurtys Point	13.4 - 19.7 (17.1 ± 2.0)	20.5 ± 4.3	60-67% (3 MPN/g)	11, 5 (5)	0% (ND)	Inter & sub-tidal	Estuarine

[1] Limited survey data available, [2] Not detected, [3] Oysters, [4] Mussels

Table 12: Assumptions used for inputs into the Oyster Refrigeration Index for post-harvest *V. parahaemolyticus* growth under AQAAC and Tasmanian VCP implementation.

Time (hrs)	ASQAAC		Time (hrs)	VCP level 1 (water temp ≤19°C, air temp ≤30°C)		Time (hrs)	VCP level 2 (water temp >19°C, air temp >30°C)	
$T_0 – T_2$	W – A °C	Out of water, 2 hrs to reach air temp	$T_0 – T_2$	W – A°C	Out of water, 2 hrs to reach air temp	$T_0 – T_2$	W – A°C	Out of water, 2 hrs to reach air temp
$T_2 – T_{22}$	A°C	20 hrs at air temp	$T_2 – T_{10}$	A°C	8 hrs at air temp	$T_2 – T_5$	A°C	3 hrs at air temp
$T_{22} – T_{24}$	A°C – 10°C	Into refrigeration & 2 hrs to cool to ASQAAC stipulated temp	$T_{10} – T_{12}$	A°C – 10°C	Into refrigeration & 2 hrs to cool to ASQAAC stipulated temp	$T_5 – T_7$	A°C – 10°C	Into refrigeration & 2 hrs to cool to ASQAAC stipulated temp
$T_{24} – T_{96}$	8°C	4-d supply chain	$T_{12} – T_{96}$	8°C	4-d supply chain	$T_{24} – T_{96}$	8°C	4-d supply chain

T=time, W=harvest water temperature, A=ambient air temperature

Table 13: Predicted *V. parahaemolyticus* growth using the Oyster Refrigeration Index post-harvest under ASQAAC and the Tasmanian VCP guidance for time into the cool chain.

Harvest water temp (°C)	Ambient air temp (°C)	ASQAAC implemented			VCP level 1 implemented			VCP level 2 implemented		
		10 cell/g	100 cell/g	1,000 cells/g	10 cells/g	100 cells/g	1,000 cells/g	10 cells/g	100 cells/g	1,000 cells/g
17	15	5	54	540	4	45	448	4	41	414
17	20	37	369	3,687	11	107	1,072	6	64	641
17	25	2,575	25,750	25,7500	74	739	7,386	17	168	1,682
17	30	18,39718	4,773,760	4,773,760	1,464	14,645	146,449	75	749	7,489
23	15	5	54	540	4	45	448	4	41	414
23	20	37	369	3,687	11	107	1,072	6	64	641
23	25	2,575	25,750	257,500	74	739	7,386	17	168	1,682
23	30	18,39718	4,773,760	4,773,760	1,464	14,645	146,449	75	749	7,489

Yellow = marginal food safety classification (<3-100 CFU/g), orange = unsatisfactory classification (100-10,000 CFU/g) and red = potentially hazardous classification (>10,000 CFU/g) for ready to eat foods by FSANZ (FSANZ, 2022).

7.3.5. Reduction steps for *V. parahaemolyticus* post-harvest

In assessing risk of *Vibrio* in oysters post-harvest, no reduction steps in *Vibrio* numbers other than temperature control were assumed as most shellfish is consumed raw. Post-harvest temperature control and good hygiene practices are assumed in mitigating risk from bacterial growth. We have not assessed the reduction in risk that may occur during post-harvest freezing, as we have no information on the volume of product that may undergo this process.

We have used the Oyster Refrigeration Index tool to estimate *V. parahaemolyticus* growth post-harvest as oysters enter the cool chain under ASQAP or VPC requirements, with a 4-day supply chain estimated. The Oyster Refrigeration Index is based on modelling which showed decreases in *V. parahaemolyticus* levels at temperature below 12.6ºC (Fernandez-Piquer et al., 2011).

7.3.6. Assessment of *V. parahaemolyticus* risk

Table 11 summarises the *V. parahaemolyticus* results for each of the 11 surveyed Tasmanian harvest areas, while **Table 14** assigns a qualitative risk assessment for elevated *V. parahaemolyticus* levels in each of the harvest areas based on the survey results (prevalence, maximum levels observed and presence of pathogenic strains), previous illnesses (sporadic and outbreak), summer water temperatures, harvest volumes and implementation of ASQAP or VCP.

Vibrio parahaemolyticus levels were assigned a high (>1,000 CFU/g), medium (100-1,00 CFU/g) or low (<100 CFU/g) rating (**Table 14**) depending on the maximum levels found during the survey using the FSANZ microbiological guidelines (FSANZ, 2022). We stress that at present the risk of elevated *V. parahaemolyticus* levels cannot be converted into the risk of human illness, as the factors determining illness and the dose required to cause illness are yet to be fully elucidated.

The presence of *tdh* and *trh* are potential indicators of pathogenicity but are by no means determinant. Cook et al (2002) detected *V. parahaemolyticus tdh*+ strains in 6% of BMS from the USA with the prevalence of *V. parahaemolyticus tdh*+ strains significantly correlated to water temperature and to the prevalence of total *V. parahaemolyticus* (Cook et al., 2002). Furthermore, the failure to detect pathogenic *V. parahaemolyticus* in shellfish more frequently was attributed to the low numbers and uneven distribution of the organism (Cook et al., 2002). Pathogenic *V. parahaemolyticus tdh*+ or *trh*+ strains have also been isolated from 6% (n=230) of BMS from Thailand and 2.9% (*tdh*+) and 10.6% (*trh*+) of seafood (fish and shellfish; n=104) from various European countries (Vuddhakul et al., 2006, Lopatek et al., 2018). As 6% was the average *V. parahaemolyticus* containing the *tdh*/*trh* genes observed in our and other international BMS investigations, we assigned a high or low rating to pathogenicity marker presence based on whether the maximum percentage found in any growing area was above or below 6% (**Table 14**). The results of our survey showed that the prevalence of *tdh*+ or *trh*+ *V. parahaemolyticus* isolates was high in many Tasmanian harvest areas, particularly in the north and north-east. The prevalence was lowest in the South East and Bruny regions, with the exception of Fluertys Point.

Water temperature was found to be the primary predictor of *V. parahaemolyticus* prevalence and levels in harvest areas state-wide. However, each harvest area had a site-specific predictive model developed based on our surveyed microbiological and environmental data for *Vibrio* at harvest (see appendices for area specific information). Other environmental data not surveyed may improve or refine these predictive models.

The Oyster Refrigeration Index tool was used to estimate post-harvest *V. parahaemolyticus* growth/decline and assist in assigning a qualitative risk for elevated *V. parahaemolyticus* levels in each growing area based on initial harvest, *V. parahaemolyticus* levels observed and average summer

maximum air temperature (approximately 20°C). The risk may vary under exceptional temperature conditions. The Oyster Refrigeration Index tool may overestimate post-harvest growth, but it may also overestimate *V. parahaemolyticus* decline at temperatures under 10°C.

Table 14: Qualitative risk analysis of Tasmanian oyster for *V. parahaemolyticus* at retail for surveyed harvest areas during average summer conditions.

Regions	Historic illness in region[1] (none/ sporadic/ outbreak)	Growing area surveyed within region	Expected summer water temperature compared with min Av temp linked to Vp in present survey	Summer Vp prevalence high <70% Medium 30-70% Low <30%	Summer max Vp levels (FSANZ levels) based on international standards High >1,000 CFU/g Medium 100-1000 CFU/g Low <100 CFU/g	Summer tdh &/or trh prevalence Low ≤6% High >6%	Harvest volume (High/Low)	Qualitative Vp level at retail if following ASQAP temp controls (L/M/H)	Mandatory Food Safety Management System (VCP)[2]	Qualitative Vp level at retail if following VCP1 (VCP2)[3] temperature controls (L/M/H)
North West	Sporadic 2019	Duck Bay	Always above	High	Medium	High	Low	M	No	M (L)
Moulting Bay	Outbreak 2016, Sporadic 2019	Moutling Bay	At or above	High	Medium	High	High	M	Yes	M (L)
Upper East Coast	Sporadic 2019	Great Oyster Bay[2]	Always above	-	-	-	Low	-	No	-
		Great Swanport	At or above	High	High	High	Low	M	Yes	M (M)
		Little Swanport	Mostly above	Medium	Medium	Low	Low	M	No	M (L)
Mid East Coast	Sporadic 2019	Boomer Bay	Mostly above	Medium/high	Low	High	High	L	No	L (L)
		Boomer Bay East	Always above	Medium/high	Low	Low	High	L	No	L (L)
South East Region	Sporadic 2019, 2022	Pipe Clay Lagoon	Mostly above	Low/medium	Low	Low	High	L	Yes	L (L)
		Pitt Water	Always above	Low/medium	Low	Low	High	L	No	L (L)
Bruny Region	Sporadic 2017, 2022	Great Bay	Sometimes above	Low/medium	Low	Low	Low	L	No	L (L)
		Fleurtys Point	Sometimes above	Medium	Low	High	Low	L	No	L (L)

[1] All illnesses occurred between January and April inclusive, only illness from commercial product included
[2] Not enough survey information gathered to enable a qualitative risk assessment to be made for Great Oyster Bay.
[3] VPC1 (when water temperature is ≤19°C and air temperature is ≤30°C), VPC2 (when water temperature is >19°C or air temperature is >30°C).

7.4. Conclusions

Levels of *V. vulnificus* were exceptionally low in Tasmanian growing areas, and no illness has previously been associated with this organism in shellfish from Tasmania. This is indicative of a low risk of vibriosis associated with Tasmanian oysters and this *Vibrio* species. The only growing area with *V. vulnificus* detected at significant levels was Great Swanport, and thus *V. vulnificus* risk is slightly elevated in this area.

It is not possible to assess the risk of vibriosis from *V. parahaemolyticus* in shellfish sourced from Tasmanian growing areas due to significant knowledge gaps in the international arena regarding factors determining pathogenicity and the lack of dose response models associated with potentially pathogenic strains. However, a qualitative assessment was made of the potential *V. parahaemolyticus* levels at retail following various post-harvest temperature regimes.

Water temperature was a major driver of *V. parahaemolyticus* levels at harvest and air temperature was a major driver of *V. parahaemolyticus* growth and decline post-harvest. In determining our qualitative risk of *V. parahaemolyticus* levels, we have assumed an average maximum summer daily temperature of 20^{o}C, based on our environmental data and supported by historic BOM data. Under these conditions, and assuming rapid cooling of shellfish to reach an internal temperature of ≤10^{o}C (as opposed to only entry into the cool chain) within the specified time frames, the Tasmanian VCP is effective at reducing *V. parahaemolyticus* levels (**Table 13**), and hence we recommend businesses consider reducing the maximum temperature threshold for the VCP to trigger entry into the cool chain within 7 hours from 30^{o}C to 20^{o}C. Under exceptional summer temperature conditions and during marine heat waves, *V. parahaemolyticus* risk could be elevated and tight temperature control is highly recommended under such conditions.

Although, water temperature is a major driver for *V. parahaemolyticus* levels in the harvest area, with levels higher in the north and north-east, other environmental factors not measured during the survey may be important as correlation with temperature alone was often low (R^2<0.50). Furthermore, some environmental data (air temperature, rainfall and river flow) collected during our survey was often relatively remote to the harvest area (see appendices). Both environmental conditions and production systems may vary within a harvest area. Also, the current Tasmanian *Vibrio* survey was only undertaken over three consecutive summer/autumn seasons between 2020 and 2022. A survey conducted during a marine heatwave period, as occurred in 2016 which coincided with first Tasmanian vibriosis outbreak, may have had a different outcome.

Although *V. parahaemolyticus* was detected in some areas during the winter/spring seasons, mainly in the north and north-east regions, the levels were very low (<3 MPN/g), pathogenic strains (*tdh*+ and/or *trh*+) were absent and no historic illnesses had been reported, suggesting vibriosis risk is low during this period. However, New Zealand unexpectedly had outbreaks of vibriosis under similar circumstances, so vigilance is needed.

During the summer/autumn period higher prevalence of *tdh*+ or *trh*+ *V. parahaemolyticus* strains were detected in some areas, higher than the average statewide prevalence and that reported for other environmental oyster surveys. The new Australian Standard (AS 5013.18.1) for *V. parahaemolyticus* is qualitative only, but does have provision for testing for these potentially pathogenic (*tdh*+/*trh*+) strains. No *V. parahaemolyticus* strain types associated with shellfish related vibriosis that has been attributed to Tasmanian oysters were detected during our limited investigation. However, we did detect a range of ST types; some of which have been linked to clinical cases or belong to clinically related clusters from other places. Whole genome sequencing MLST typing has become more accessible and affordable in recent

years and should be considered in routine surveillance or at least during trace back of foods. It is concerning that some of our ST types have been shown to contain single or multiple antibiotic drug resistance which is of relevance when treating clinical infections.

7.5. Recommendations

1. *Vibrio* pose a food safety risk in shellfish harvested during the summer/autumn seasons. *Vibrio* may be present in shellfish harvested from water temperatures as low as 11-15ºC, with risk increasing as water temperatures increase and potentially during marine heat waves. *Vibrio* Control Plan temperature controls should be reviewed in the light of the results in this report.

2. All Tasmanian oyster growers should be implementing a VCP during summer months. We recommend growers in the north-west, Moulting Bay and the upper-east coast implement level 2 temperature requirements of the VCP during the summer months to further reduce *V. parahaemolyticus* levels.

3. We recommend the level 2 requirements in the VCP be adapted such that it is implemented when air temperatures are at or above 20ºC (as opposed to 30ºC as is currently stipulated).

4. Triggers and alerts for relevant water temperatures (which may be growing area specific) to implement VCP should be added to the recently commissioned eagle.io display (Oysters Tasmania sensor network). The sensor network measures real time water temperature and salinity in each of the growing areas.

5. Businesses should be aware of the time required to reduce the internal temperature of oysters to ≤10ºC and maximise the efficiency of their cooling regimes.

6. Risk communication of *Vibrio* risk needs to be provided to consumers and recreational fishers. This is a shared responsibility for businesses (farm gate sales and retail) and risk managers (recreational fishers). Consistent and non-alarmist messaging is warranted.

7. Businesses and risk managers should follow the Bureau of Meteorology forecasts for marine heat waves[4] to be forewarned of periods of elevated temperature.

8. Further research should be conducted into the genetic variations between environmental and clinical strains to aid in identification of pathogenic markers and assessment of risk.

9. When *Vibrio* testing occurs, the purpose of the testing should determine the method of analysis and whether this is qualitative, quantitative and whether it includes pathogenicity markers and/or MLST typing.

10. Quantitative *Vibrio* testing, preferably with pathogenicity markers and MLST typing, should be done during outbreak events to collect information of *V. parahaemolyticus* levels in foods associated with illness.

11. *Vibrio parahaemolyticus* isolates should be collected (clinical and food) during vibriosis events and an Australian isolate collection curated and maintained.

[4] http://www.bom.gov.au/oceanography/oceantemp/sst-outlook-map.shtml

12. Post-harvest *Vibrio* growth models should be reviewed for Australian commercial oyster species.

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Appendices

Appendix 1: *Vibrio* Survey Duck Bay, 2020-2022

Background

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Duck Bay (Figure 1).

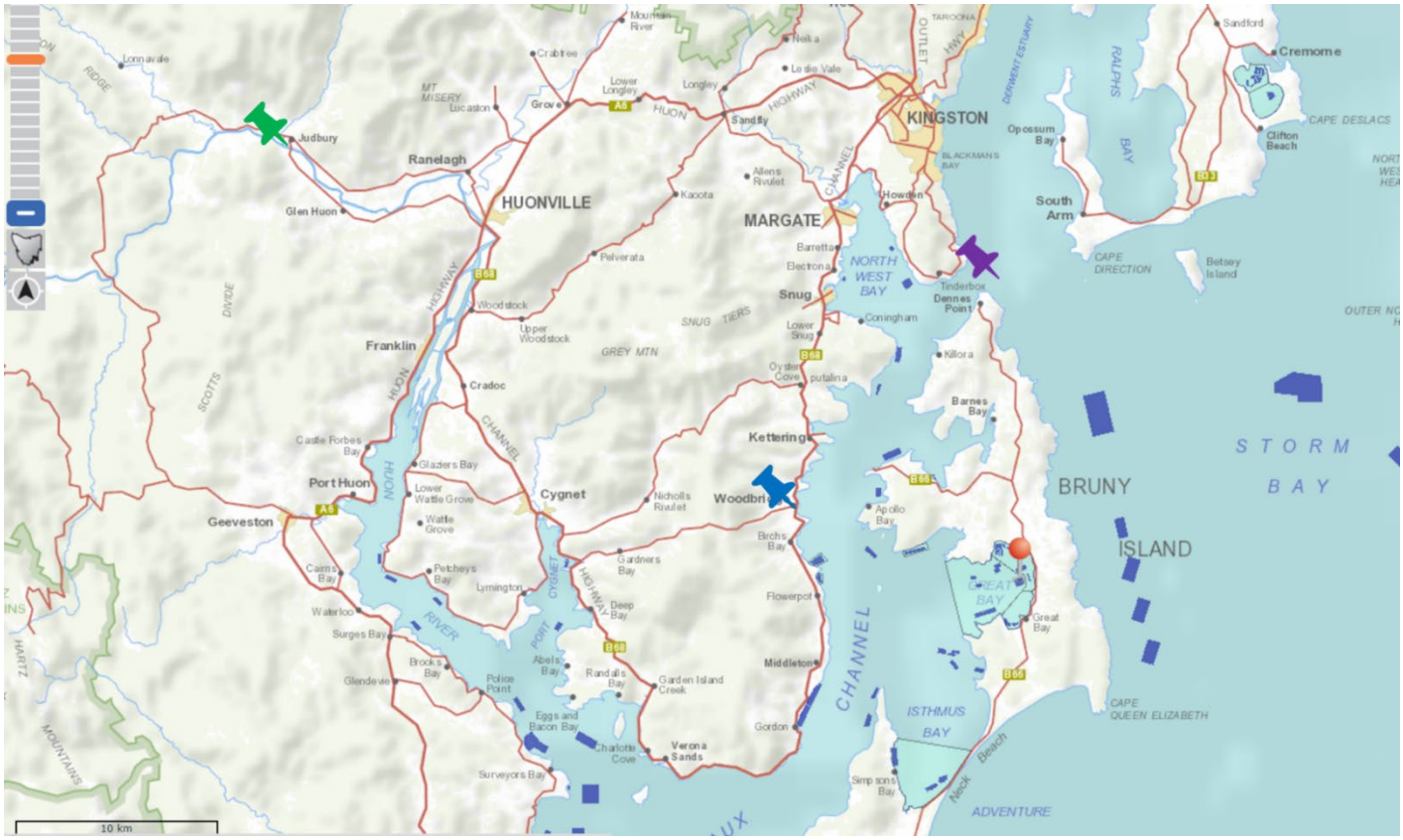

Figure 1: Duck Bay harvest area. Orange pin indicates lease from which sampling occurred. Green pin indicates Duck River flow station at Scotchtown Rd. Purple pin indicates BOM temperature station at Dennes Point. Blue pin indicates BOM rainfall station at Woodbridge. Scale bar = 10 km.

Methodology

Summary of survey design

Oysters were sampled over three summer/autumn (November to April) and two winter/spring (May to October) periods from January 2020 to end of April 2022. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data collected: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data collected: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity, water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation. Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station number 91292; Smithton Aerodrome). Maximum river flow on the day of sampling was also accessed from the

Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable, for the river identified in the harvest areas' sanitary survey. For Duck Bay the river identified was the Duck River. Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 91292; Smithton Aerodrome).

Statistical analysis

Statistical analysis of biological and environmental data was done in Primer 7 + PERMANOVA (Primer-e). Correlation among variables was investigated using draftsman's plots. *V. parahaemolyticus* (MPN/g) data were $\log_{10}(X+1)$ transformed and used to create a Euclidean distance resemblance matrix. The environmental data was also transformed if not normally distributed: rainfall data for Duck Bay was square root transformed, while river flow data was fourth root transformed. The DISTLM (distance-based linear model) routine was used to investigate the relationship between the *V. parahaemolyticus* Euclidean distance resemblance matrix and the environmental (temperature, salinity and rainfall) predictor variables. The step-wise "selection procedure" was used with the Akaike Information Criterion corrected for small sample sizes (AICc) "selection criterion" and 9999 permutations. AICc is a mathematical method for evaluating how well a model fits the data it was generated from. The output of this analysis gives a statistical marginal test for each individual predictor variable considered alone, and the best overall solution. The best linear model was visually represented using dbRDA (distance-based redundancy analysis). The formula for the resulting predictive model was generated using the regression function in excel.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Salinity and rainfall data during the survey period are shown in Figure 2A. Rainfall events were consistently observed across all seasons. Maximum flow of the Duck River was observed in the winter/spring seasons (Figure 2B). Figure 2C shows *V. parahaemolyticus* levels, sampling water temperature and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Temperature and *V. parahaemolyticus* numbers increased during summer/autumns months. Salinity in Duck Bay was consistent (29-36 PSU) but trending lower when increased river flow was observed.

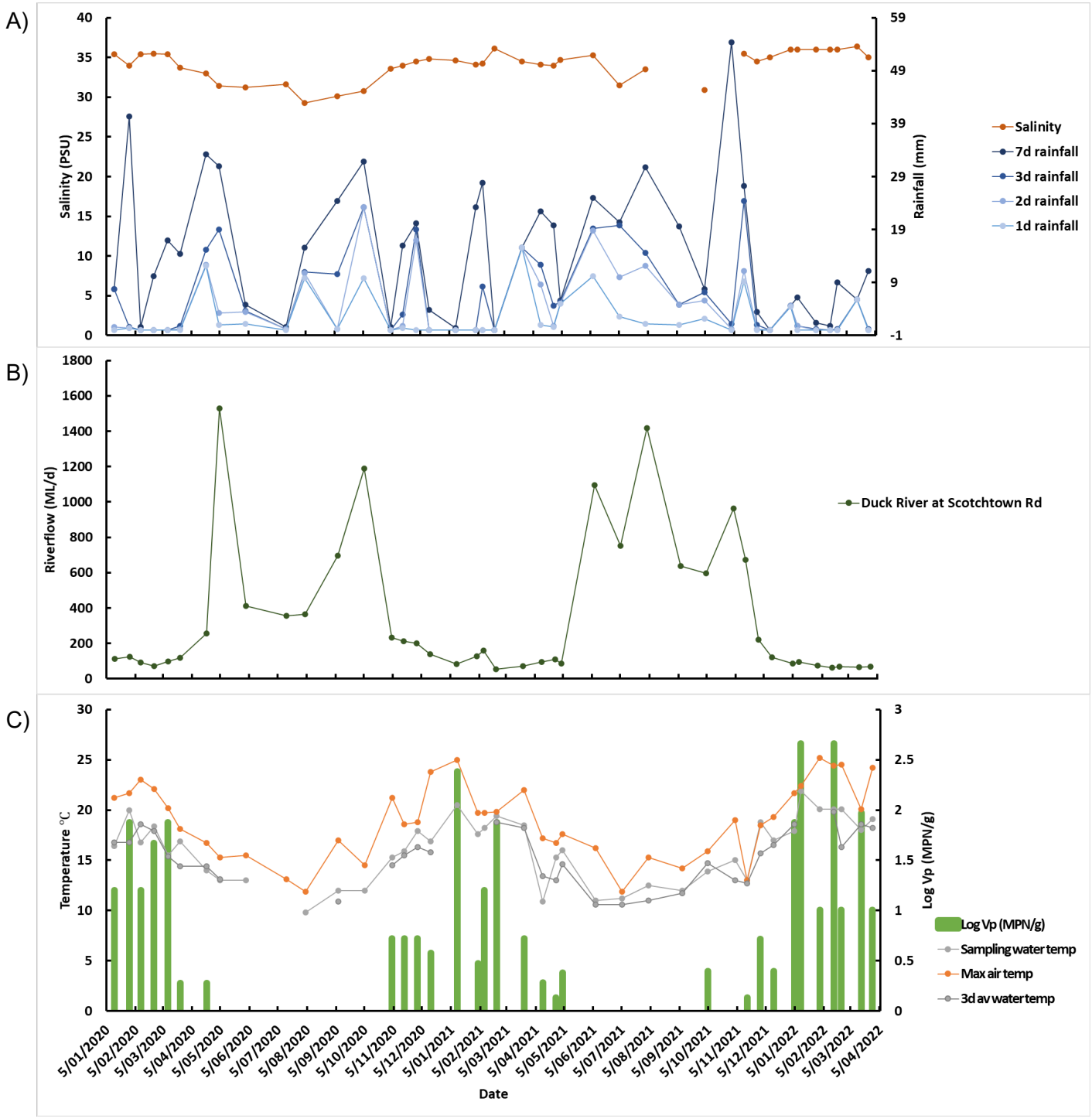

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Duck Bay. 2A shows water salinity at sampling and rainfall (1, 2, 3 and 7 days) prior to sampling. 2B shows maximum river flow 24 hours prior to sampling. 2C shows levels of *V. parahaemolyticus* detected and are overlayed with sampling water temperature, three-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence for Duck Bay broken down to the 5 seasons sampled during the survey. A higher prevalence of *V. parahaemolyticus* was consistently detected during the summer/autumn periods (91-100%), although it was also detected in the winter/spring of 2021 (33% prevalence). *V. parahaemolyticus* strains containing the *tdh* (9-18%) and *trh* (9-57%) were only detected during summer months, although *V. parahaemolyticus* was detected all year round.

No *V. vulnificus* was detected during the survey.

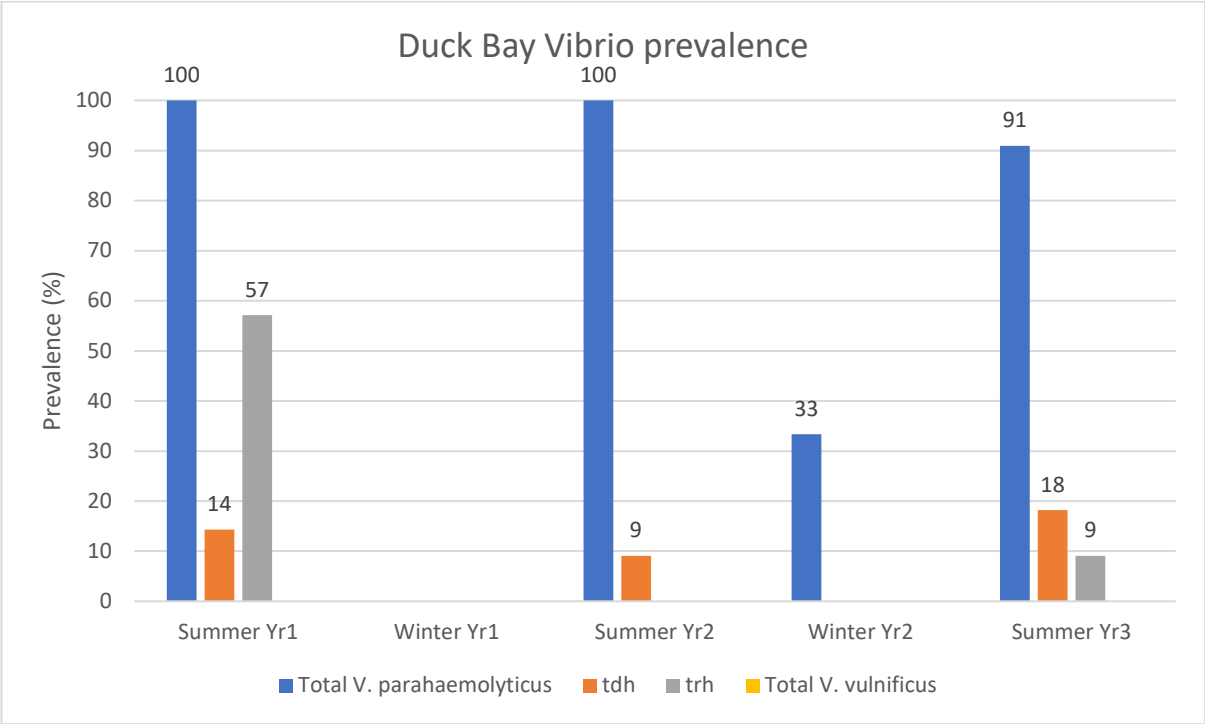

Figure 3: Seasonal *Vibrio* prevalence during the survey period.

No *V. parahaemolyticus* was detected when sampling water was <10°C or when the average, minimum and the maximum water temperatures 3-day prior to sampling were, <12°C, 4°C and <15°C, respectively. No relationship could be established with *V. parahaemolyticus* and sampling of shellfish either above or below the water. Noting that most oysters from Duck Bay were collected (n=22/27) above the water, n=16 of these were positive for *V. parahaemolyticus*, while n=2/5 collected below the water were also positive for *V. parahaemolyticus.* No clear relationship between tidal stage and *V. parahaemolyticus* levels could be deduced (Figure 4).

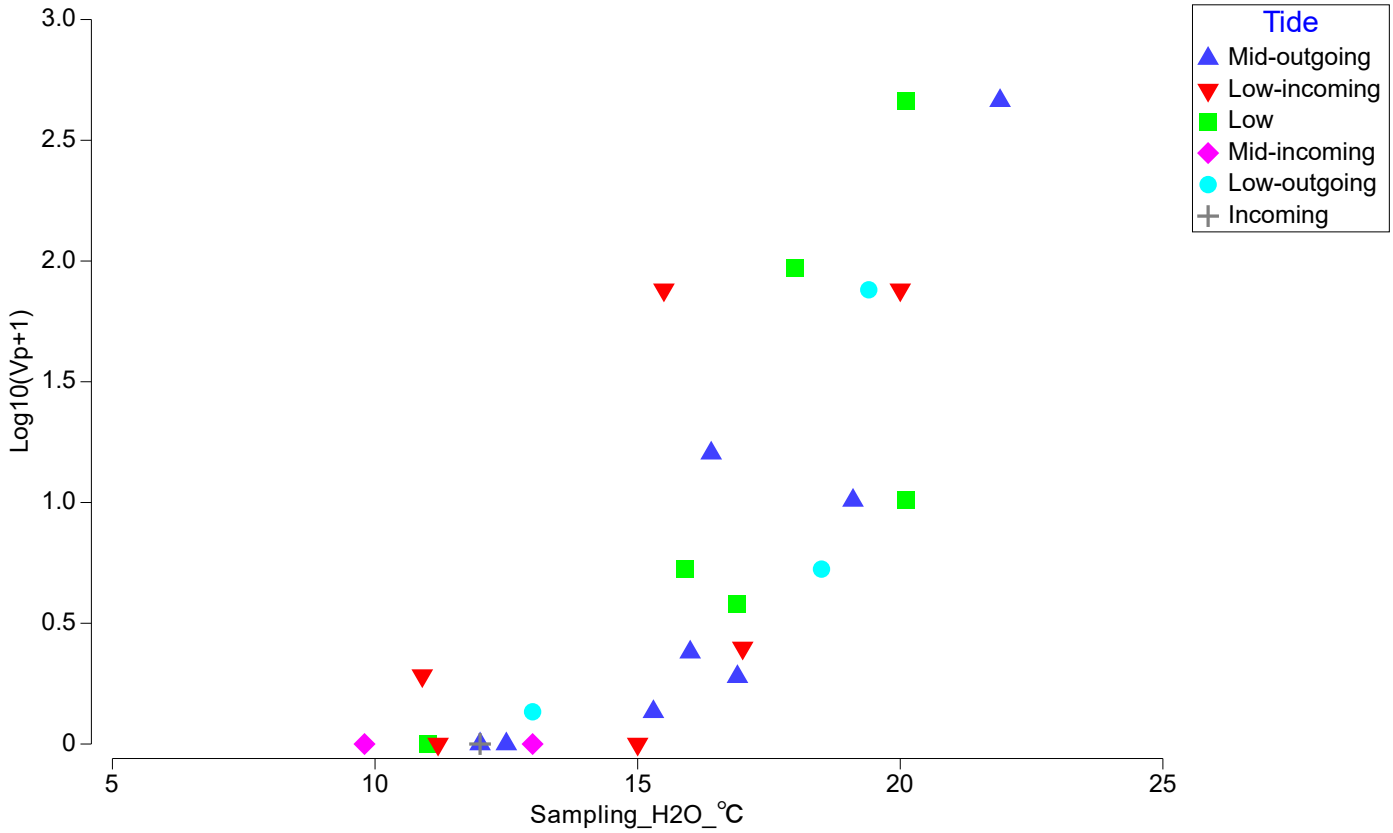

Figure 4: Scatter plot of *V. parahaemolyticus* versus sampling temperature identified by tidal stage of collection.

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data during the survey aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of environmental conditions were recorded in the growing area over the period of the survey. *V. parahaemolyticus* numbers where low to moderate with a maximum detection of 460 MPN/g.

Table 1: Summary table of environmental parameters over the summer/autumn & winter/spring survey periods.

	Summer (n=18)					**Winter (n=9)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	10.9	21.9	17.2	2.8	18	9.8	16.0	12.2	1.8	8
Sampling air temp	11.4	22.0	16.7	3.8	11	9.0	15.0	11.5	2.3	5
3-day min H_2O temp	4.5	15.5	10.3	3.1	16	4.5	13.0	7.1	3.4	5
3-day max H_2O temp	15.0	29.0	21.1	4.0	16	13.0	17.0	15.0	1.5	5
3-day Av H_2O temp	12.7	19.8	16.1	2.3	16	10.6	14.6	11.7	1.7	5
1-day max air temp	13.0	25.2	20.4	3.1	18	11.9	17.6	14.5	1.9	8
Salinity (PSU)										
Sampling H_2O	33.7	36.4	35.1	0.9	17	29.3	35.3	32.2	2.1	7
Rainfall (mm)										
1-day	0	15.6	1.8	4.2	18	0	10.2	4.5	4.3	8
2-days	0	15.6	2.5	4.6	18	0.2	23.2	9.8	7.5	8
3-days	0	24.4	4.3	6.8	18	0.2	23.2	11.3	8.3	8
7-days	0	54.4	14.7	14.6	18	0.6	31.8	17.1	11.4	8

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods.

***Vibrio* enumeration (MPN/g)**						
Vibrio	**Summer (n=18)**			**Winter (n=9)**		
	Min	**Max**	**Positive (n=)**	**Min**	**Max**	**Positive (n=)**
Total *V. parahaemolyticus*	0	460	17	0	1.4	1
tdh	0	0.92	2	0	0	0
trh	0	1.5	3	0	0	0
Total *V. vulnificus*	0	0	0	0	0	0

Statistical analysis

Temperature

All the temperature predictor variables were highly inter-correlated (R>0.70), including the various water and maximum daily air temperatures (Figure 5). The highest correlations were observed between average water temperature 3-days before sampling, and with either sampling water temperature or maximum water temperature 3-days before sampling (R=0.91). *V. parahaemolyticus* levels in Duck Bay oysters were highly correlated with all the temperature predictor variables (R<0.69). The highest correlations were observed with the maximum water temperature, followed by the average water temperature, 3-days before sampling.

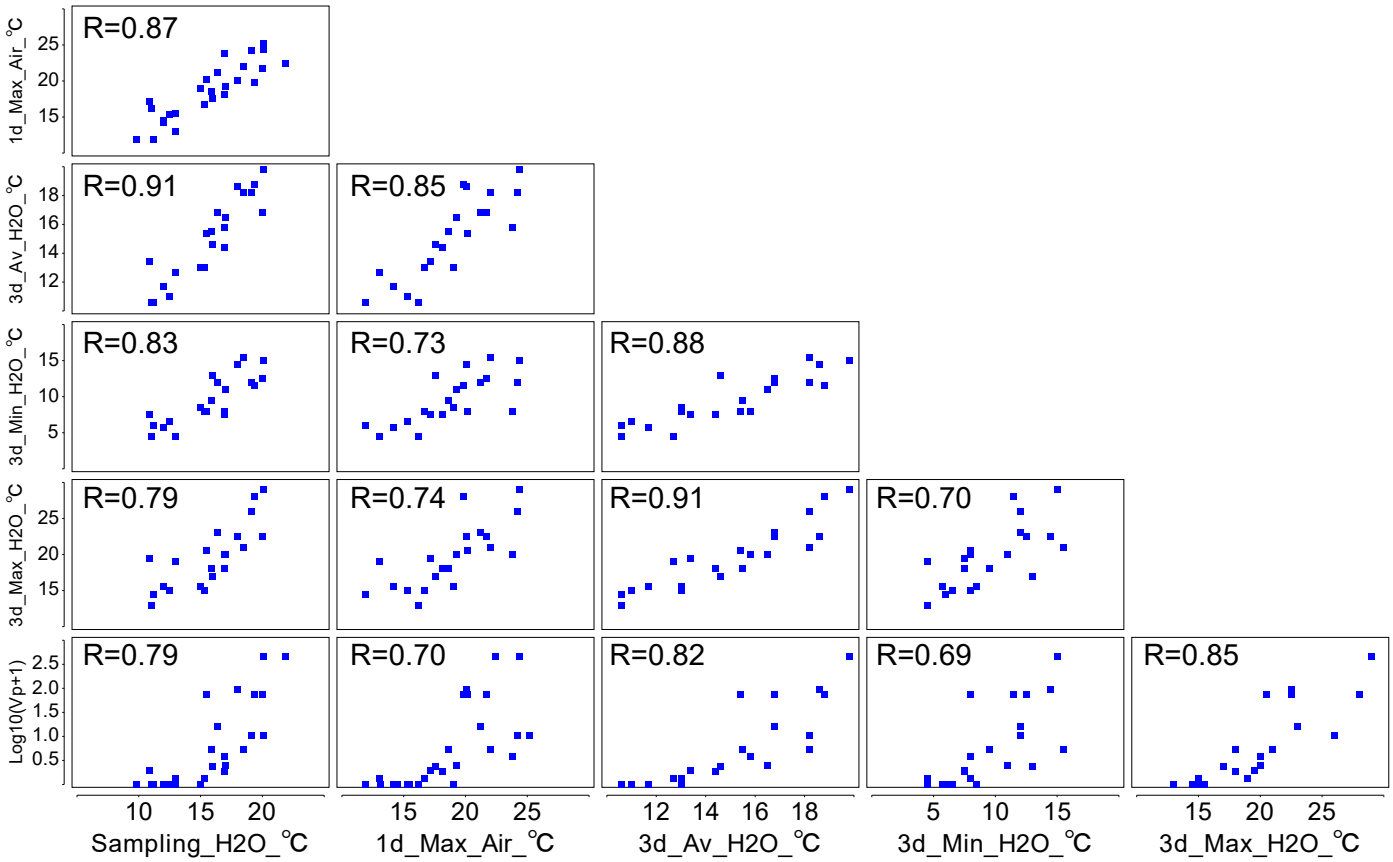

Figure 5: Draftsman's plots of temperature variables and *V. parahaemolyticus*.

Rainfall

Rainfall 1- to 7-days before oyster sampling were positively correlated (Figure 6). The strongest inter-rainfall correlations were observed between 2- and 3-day rainfall (R=0.94), followed by 1- and 2-day rainfall. *V. parahaemolyticus* levels were weakly negatively correlated with rainfall; the highest correlation between *V. parahaemolyticus* levels and rainfall was observed with the 3-day rainfall (R=-0.51).

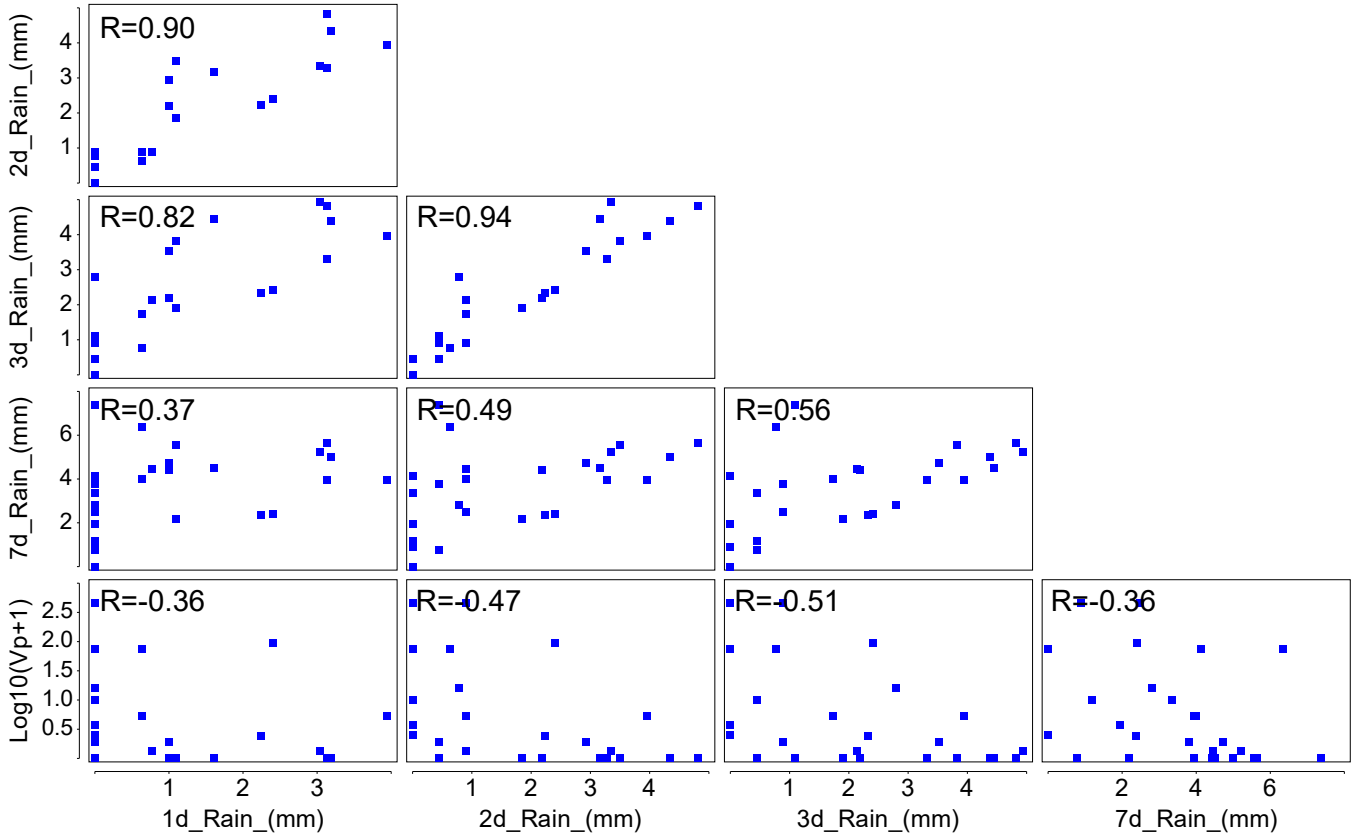

Figure 6: Draftsman's plots of rainfall variables and *V. parahaemolyticus*. Rainfall data was square root transformed.

Salinity and river flow

Duck Bay harvest water salinity and maximum river flow of the Duck River were negatively correlated (Figure 7). V. *parahaemolyticus* levels were positively correlated with harvest water salinity and negatively correlated with maximum river flow of the Duck River.

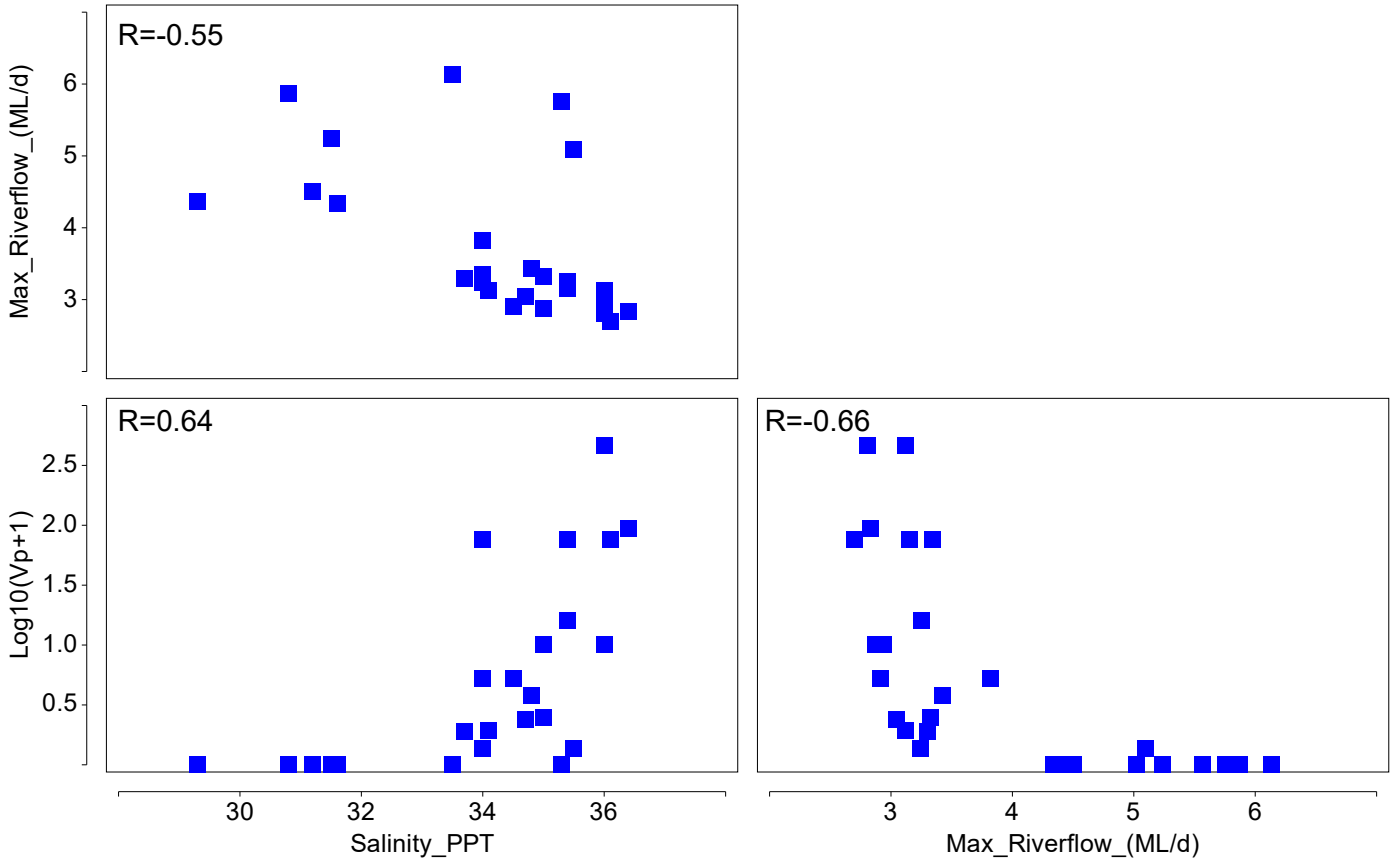

Figure 7: Draftsman's plots of salinity, river flow and *V. parahaemolyticus*. River flow data was fourth root transformed.

Linear model generation for *V. parahaemolyticus*

The marginal statistical tests in DISTLM showed significant relationships of all temperature predictor variable with *V. parahaemolyticus* levels; sampling water temperature (Pseudo-F=19.7, P<0.001), maximum daily air temperature (Pseudo-F=14.4, P=<0.001), 3-day average water temperature prior to sampling (Pseudo-F=31.6, P<0.001) and minimum (Pseudo-F=14.0, P=0.001) and maximum (Pseudo-F=38.4, P<0.001) water temperature 3 days prior to sampling, individually explaining 54%, 46%, 65%, 45% and 69% of the explained variability. It should be noted that all these temperature variables were highly inter-correlated. The marginal statistical tests in DISTLM also showed significant relationships between salinity (Pseudo-F=9.3, P=0.008), maximum river flow (Pseudo-F=9.6, P=0.007), 2-day (Pseudo-F=6.6, P=0.019) and 3-day rainfall (Pseudo-F=9.2, P=0.008), and *V. parahaemolyticus* numbers, individually explaining 35%, 36%, 28% and 35% of the explained variability. In the most accurate model selection maximum water temperature 3-days prior to sampling described 100% of the explained variability in the fitted model, 69% of the total variability in the data cloud (AICc=-26.01 R^2=0.69) (Figure 8).

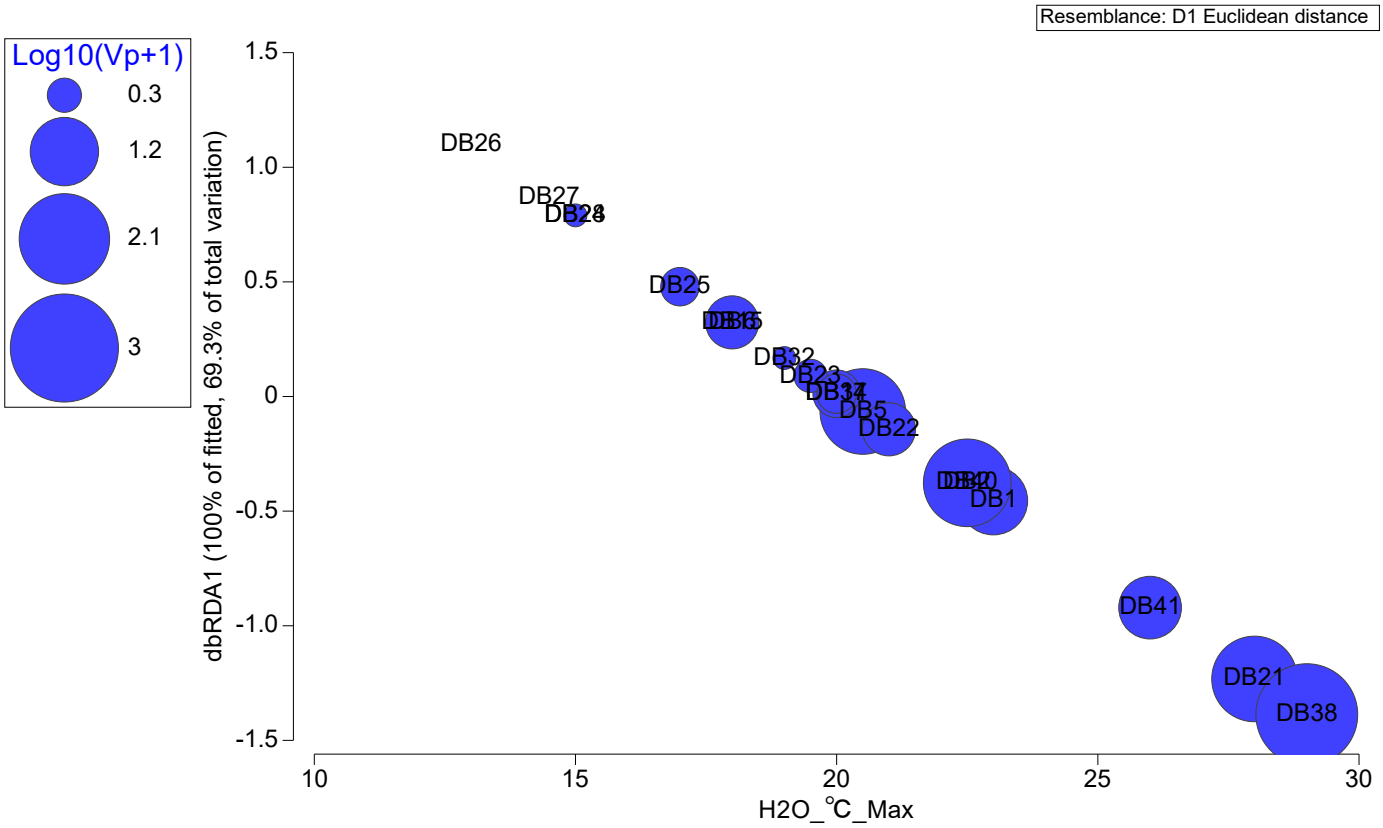

Figure 8: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with predictor variable maximum water temperature 3-days prior to sampling. Bubbles on plot indicate level of *V. parahaemolyticus* (Vp) detected in oysters sampled from Duck Bay.

The predictive model developed for *V. parahaemolyticus* in Duck Bay for oysters was based on the maximum water temperature 3-days prior to sampling:

$$y = -2.2746 + 0.15556x_0$$

Where:

y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)

x_0 = maximum water temperature 3 days prior to sampling (°C)

R^2 = 0.69

Conclusions

- *V. parahaemolyticus* was detected in Duck Bay in all three surveyed summer/autumn sampling seasons with a prevalence of 91-100%. *V. parahaemolyticus* was also detected in the second winter/spring sampling period with a prevalence of 33%.
- Levels of *V. parahaemolyticus* detected during the survey period were low to moderate; ≤460 MPN/g of oyster.
- *V. parahaemolyticus* carrying the *tdh* and *trh* genes (often associated with clinical strains) were only detected in the summer/autumn seasons with a prevalence of 9-18% for *tdh* and 9-57% for *trh*.
- *V. vulnificus* was not detected in Duck Bay during the survey period between 2020 and 2022.
- Temperature, salinity, maximum river flow and rainfall were all individually significant indicators of *V. parahaemolyticus* risk in oysters from Duck Bay. No *V. parahaemolyticus* was detected when sampling water was <10°C or when the average and the maximum water temperatures 3-day prior to sampling were, <12°C and <15°C, respectively.
- The best predictive model developed for *V. parahaemolyticus* risk in Duck Bay was based on maximum water temperature 3-days prior to sampling.

$$y = -2.2746 + 0.15556x_0$$

Where:
y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = maximum water temperature 3 days prior to sampling (°C)
R^2 = 0.69

Appendix 2: *Vibrio* Survey Moulting Bay, 2020-2022

Background

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Moulting Bay (Figure 1).

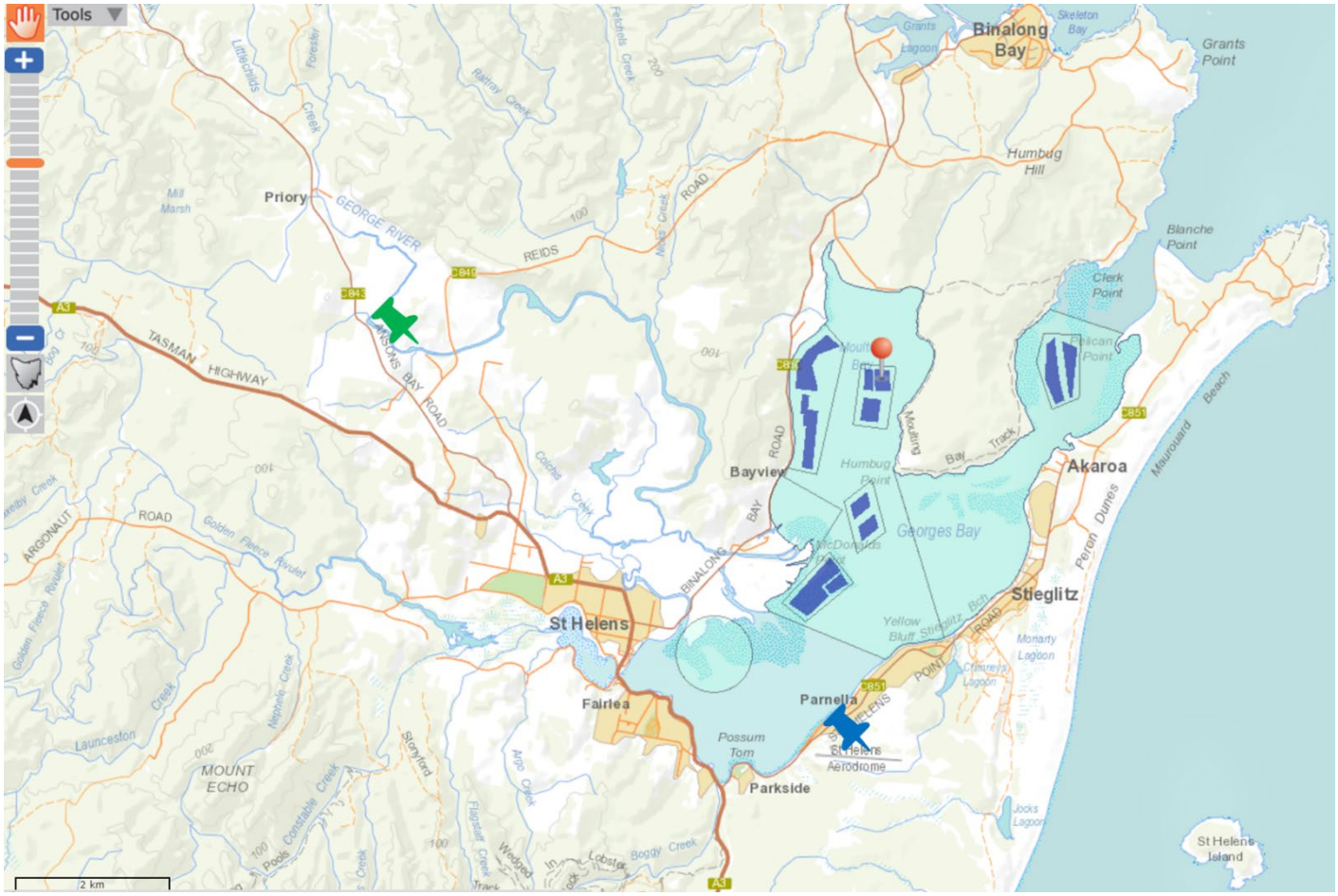

Figure 1: Moulting Bay harvest area. Orange pin indicates lease from which sampling occurred. Green pin indicates Georges River flow station. Blue pin indicated BOM weather station at St Helens Aerodrome. Scale bar = 2 km.

Methodology

Summary of survey design

Oysters were sampled over three summer/autumn (November to April) and two winter/spring (May to October) periods from January 2020 to end of April 2022. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data collected: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data collected: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity, water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation. Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station number 92120; St Helens Aerodrome). Maximum river flow on the day of sampling was also accessed from the

Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable, for the river identified in the harvest area's sanitary survey. For Moulting Bay this was the Georges River. Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 92120; St Helens Aerodrome).

Statistical analysis

Statistical analysis of biological and environmental data was done in Primer 7 + PERMANOVA (Primer-e). Correlation among variables was investigated using draftsman's plots. *V. parahaemolyticus* (MPN/g) data were $\log_{10}(X+1)$ transformed and used to create a Euclidean distance resemblance matrix. The environmental data was also transformed if not normally distributed: rainfall data for Moulting Bay was fourth root transformed and the river flow data was square root transformed. Where missing data existed in the dataset (<5%) and predominantly within the temperature dataset, the "missing" function in Primer 7 was used to estimate the value using the expectation-maximisation (EM) algorithm. The DISTLM (distance-based linear model) routine was used to investigate the relationship between the *V. parahaemolyticus* Euclidean distance resemblance matrix and the environmental (temperature, salinity, rainfall and river flow) predictor variables. The step-wise "selection procedure" was used with the Akaike Information Criterion corrected for small sample sizes (AICc) "selection criterion" and 9999 permutations. AICc is a mathematical method for evaluating how well a model fits the data it was generated from. The output of this analysis gives a statistical marginal test for each individual predictor variable considered alone, and the best overall solution. The best linear model was visually represented using dbRDA (distance-based redundancy analysis). The formula for the resulting predictive model was generated using the regression function in excel.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Salinity and rainfall data during the survey period are shown in Figure 2A. The highest rainfall events were recorded in summer months and corresponded with increased river flow of the George River (Figure 2B). Figure 2C shows *V. parahaemolyticus* levels, sampling water temperature and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Temperature and *V. parahaemolyticus* numbers increased during summer/autumns months. Salinity in Moulting Bay was variable (25-37 PSU) and drops in salinity correspond to increased rainfall and river flow.

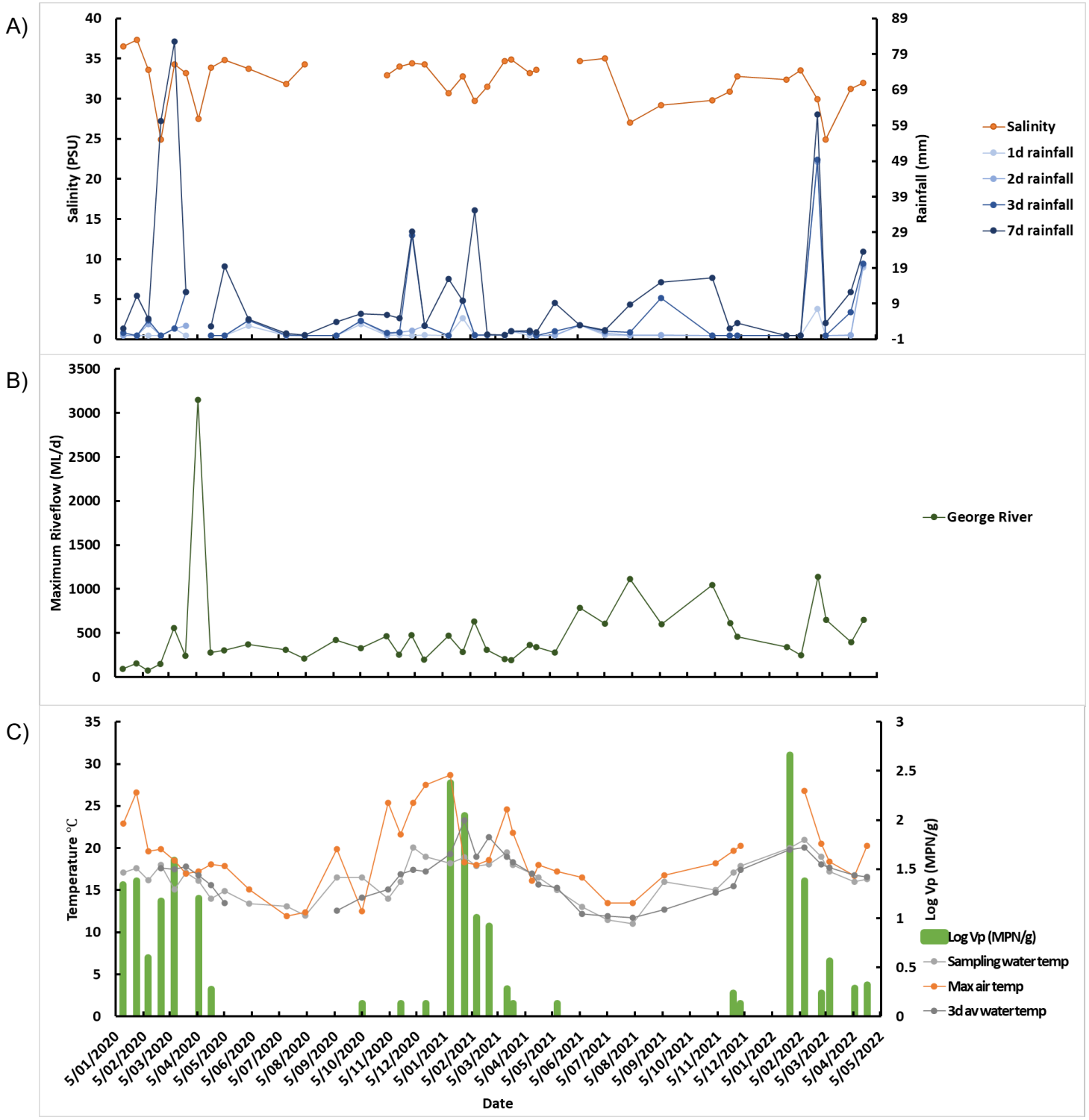

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Moulting Bay. 2A shows water salinity at sampling and St. Helens rainfall (1, 2, 3 and 7 days) prior to sampling. 2B shows maximum river flow 24 hours prior to sampling. 2C shows levels of *V. parahaemolyticus* detected and are overlayed with sampling water temperature, three-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence for Moulting Bay broken down to the 5 seasons sampled during the survey. A higher prevalence of *V. parahaemolyticus*, *tdh* and *trh* were found during summer months, although *V. parahaemolyticus* was detected all year round. *V. vulnificus* was only detected in the summer/autumn of year 3.

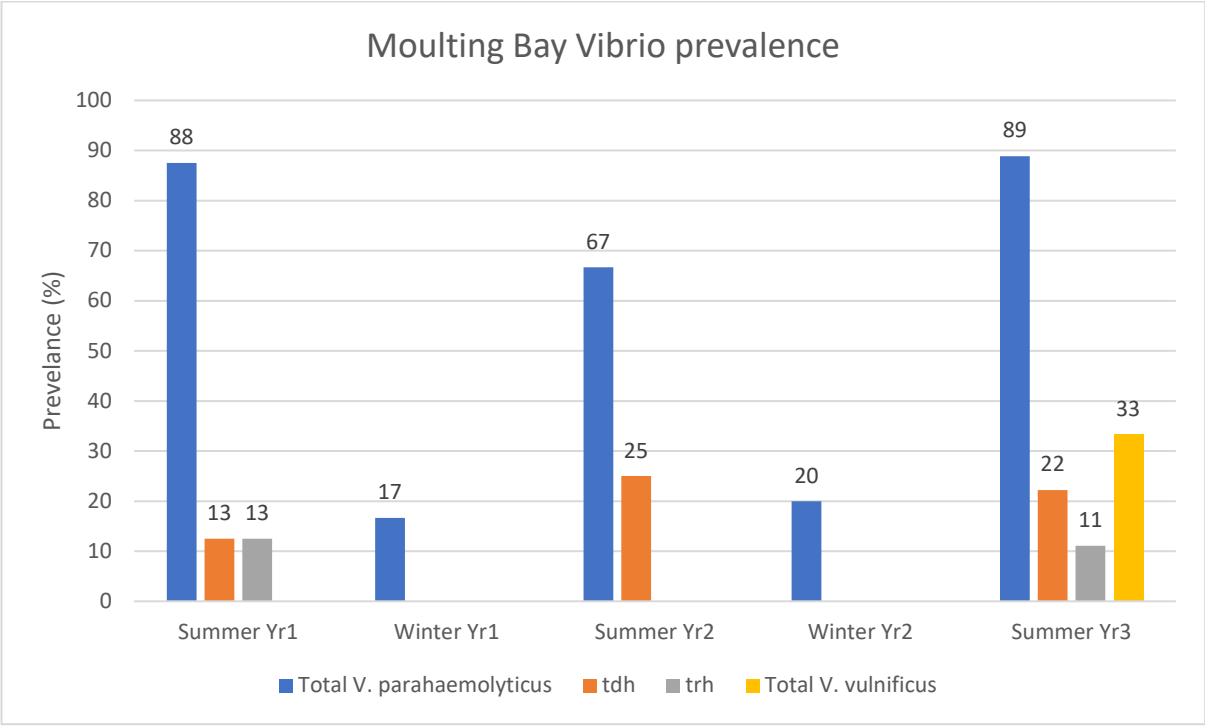

Figure 3: Seasonal *Vibrio* prevalence during the survey period

V. parahaemolyticus was not detected when sampling water temperature was <14°C, when the 3-day average water temperature was <15°C or minimum water temperature 3-days prior to sampling was <13°C. All samples were collected below water. No relationship could be deduced between tidal stage and *V. parahaemolyticus* detection (Figure 4).

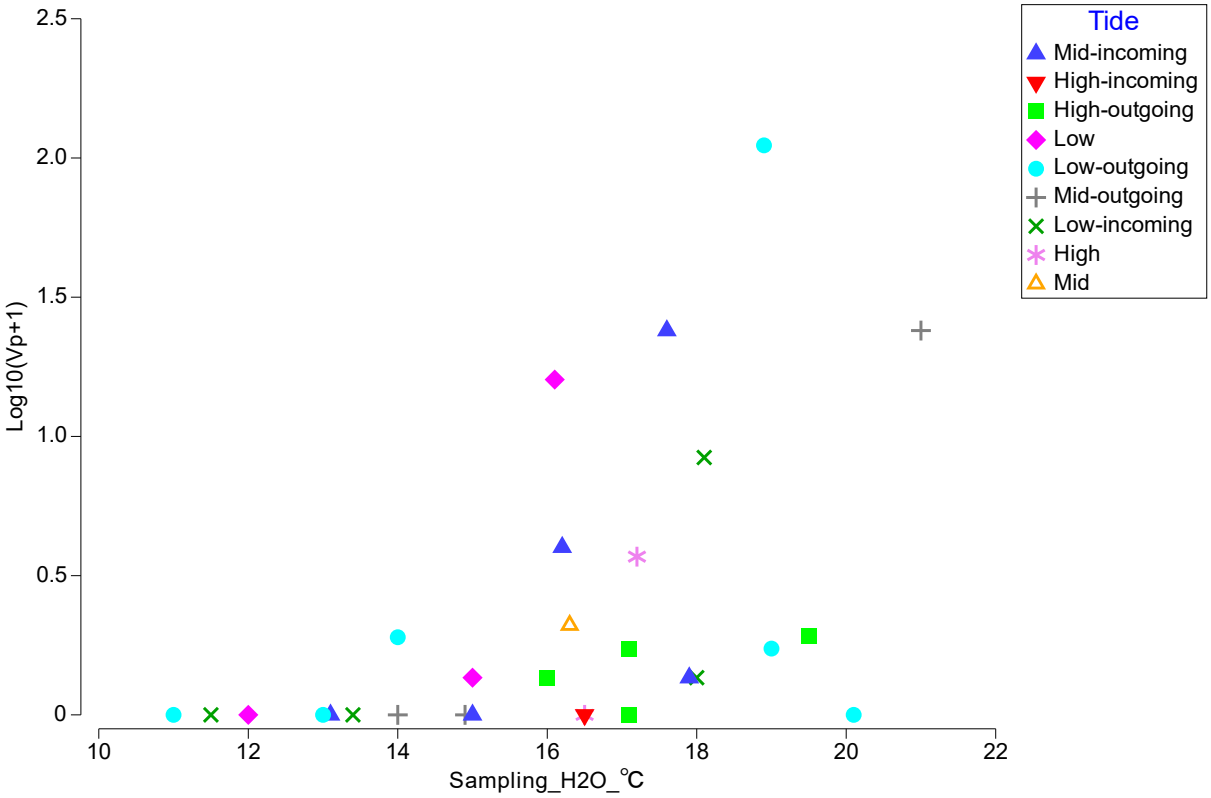

Figure 4: Scatter plot of *V. parahaemolyticus* versus sampling temperature identified by tidal stage of collection.

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data during the survey aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of environmental conditions were recorded in the growing area over the period of the survey. *V. parahaemolyticus* numbers where low, with a maximum detection of 110 MPN/g.

Table 1: Summary table of environmental parameters over the summer/autumn and winter/spring survey periods

	Summer (n=21)					**Winter (n=9)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	14.0	21.0	17.3	1.8	21	11.0	16.5	13.4	1.8	9
Sampling air temp	16.0	16.0	16.0	NA	1	NA	NA	NA	NA	0
3-day min H_2O temp	13.0	20.0	16.1	2.1	19	10.5	13.5	11.9	1.2	6
3-day max H_2O temp	16.0	23.0	19.0	2.3	18	12.5	18.5	14.6	2.2	6
3-day Av H_2O temp	14.7	23.3	17.6	2.2	19	11.7	15.3	12.9	1.4	6
1-day max Air temp	16.1	26.8	20.6	3.3	21	11.9	19.9	15.3	2.8	9
Salinity (PSU)										
Sampling H_2O	24.9	37.3	32.4	2.7	21	27.0	35.0	33.0	2.9	7
Rainfall (mm)										
1-day	0	19.2	1.7	4.6	21	0	3.0	0.7	1.3	9
2-days	0	48.8	4.5	11.4	21	0	4.4	0.9	1.6	9
3-days	0	49.4	6.4	12.7	21	0	4.4	1.2	1.5	9
7-days	0	62.0	9.8	14.7	21	0.2	19.4	5.7	6.1	9
River flow (ML/d)										
George River	75.4	3152.5	552.3	655.6	21	212.8	1114.7	489.5	295.5	9

NA = not applicable

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods

***Vibrio* enumeration (MPN/g)**						
Vibrio	**Summer (n=21)**			**Winter (n=9)**		
	Min	**Max**	**Positive (n=)**	**Min**	**Max**	**Positive (n=)**
Total *V. parahaemolyticus*	0	110	15	0	0.36	1
tdh	0	0.36	3	0	0	0
trh	0	0.36	1	0	0	0
Total *V. vulnificus*	0	0.94	2	0	0	0

Statistical analysis

Temperature

The temperature predictor variables were highly correlated (<R=0.80) with one another except for maximum daily air temperature and the water temperature measures (Figure 5). *V. parahaemolyticus* numbers were most correlated with the 3-day average water temperature prior to shellfish sampling.

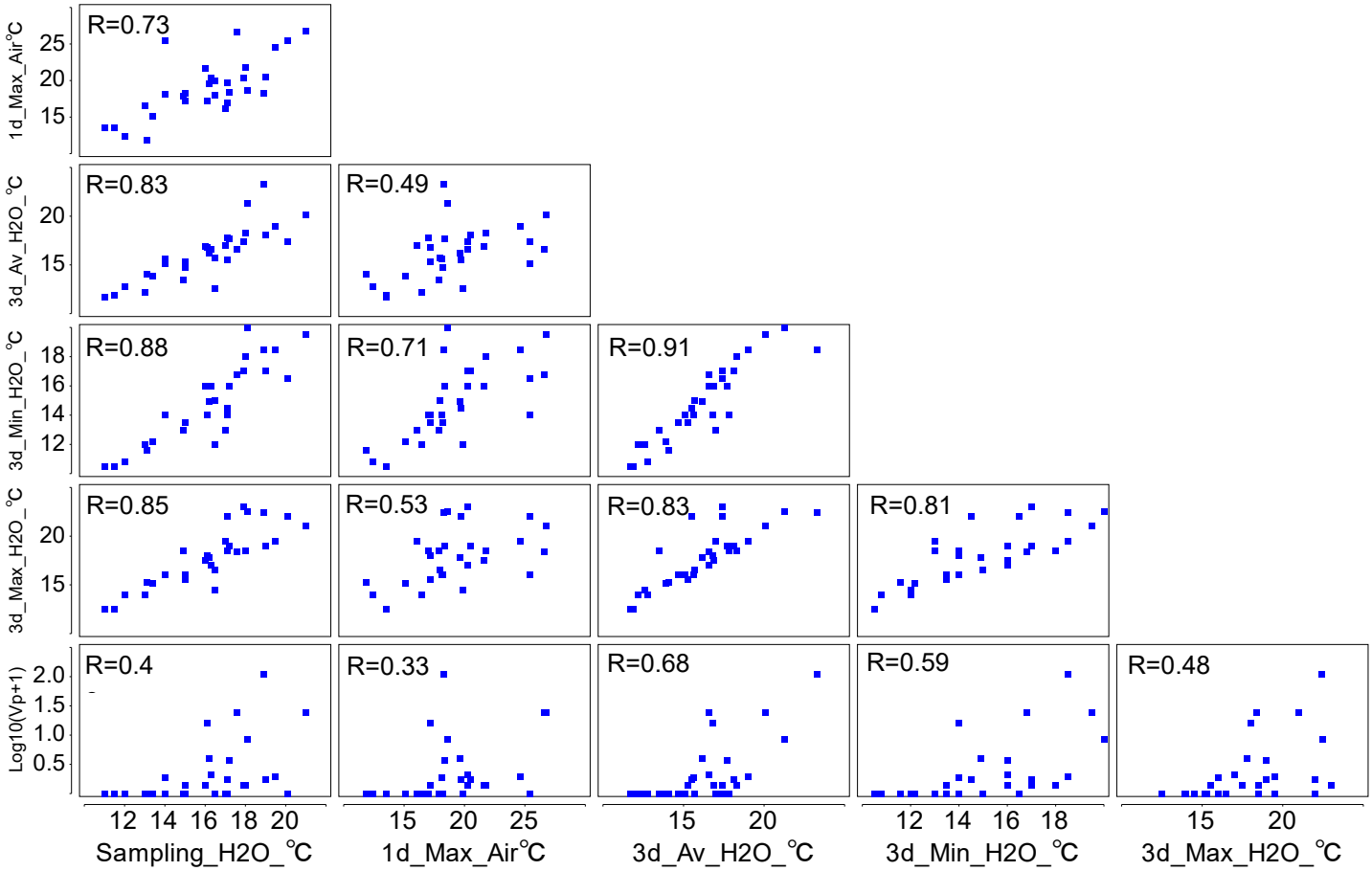

Figure 5: Draftsman's plots of temperature variables and *V. parahaemolyticus*.

Rainfall

The rainfall measures were correlated with one another with the highest correlation observed between one- and two-days rainfall and between two- and three-days rainfall (Figure 6). Poor correlation was observed between *V. parahaemolyticus* numbers and rainfall.

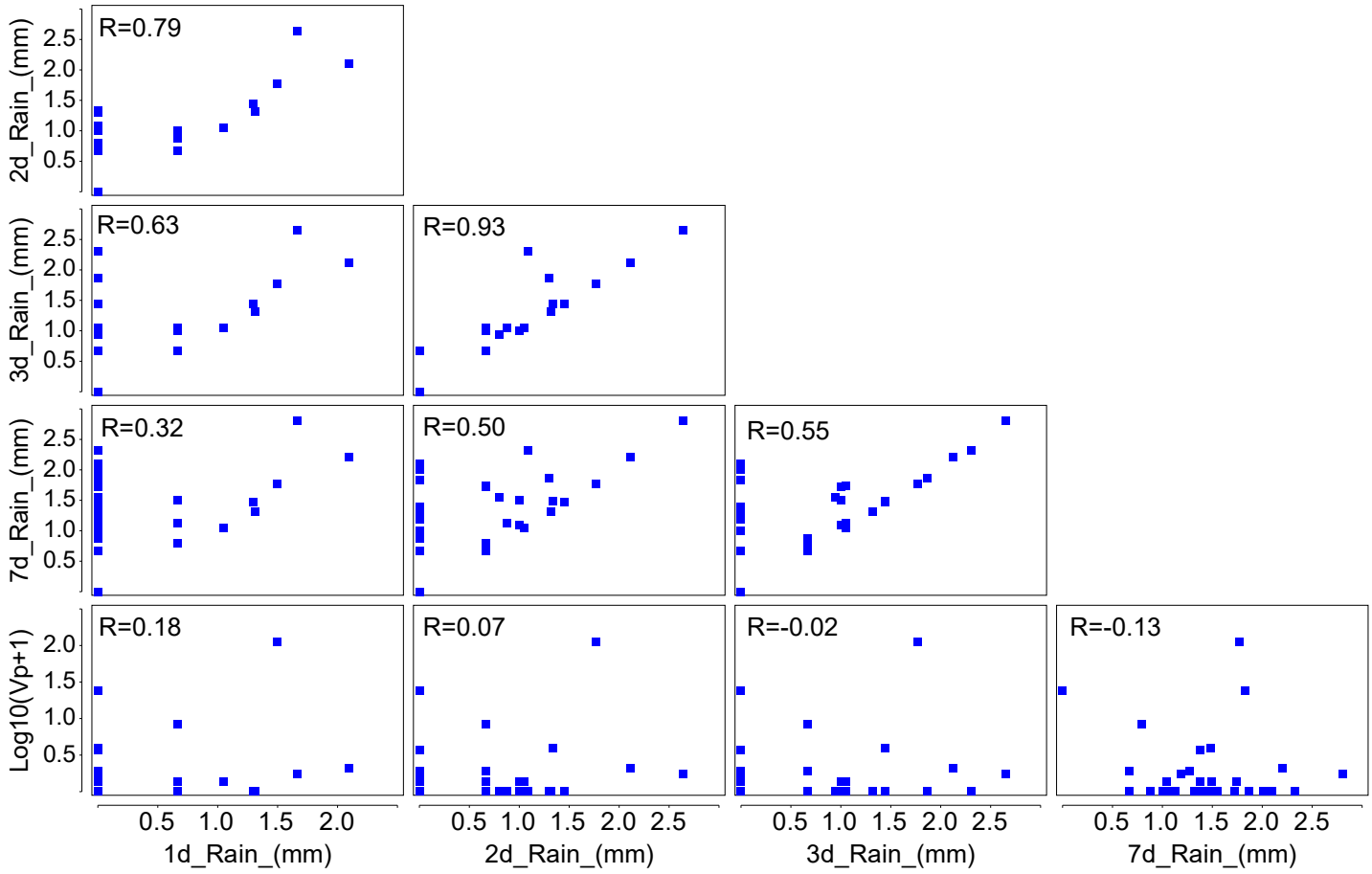

Figure 6: Draftsman's plots of rainfall variables and *V. parahaemolyticus*.

Salinity and river flow

Maximum river flow and sampling water salinity were negatively correlated (Figure 7). *V. parahaemolyticus* numbers were not correlated with either sampling water salinity or maximum river flow.

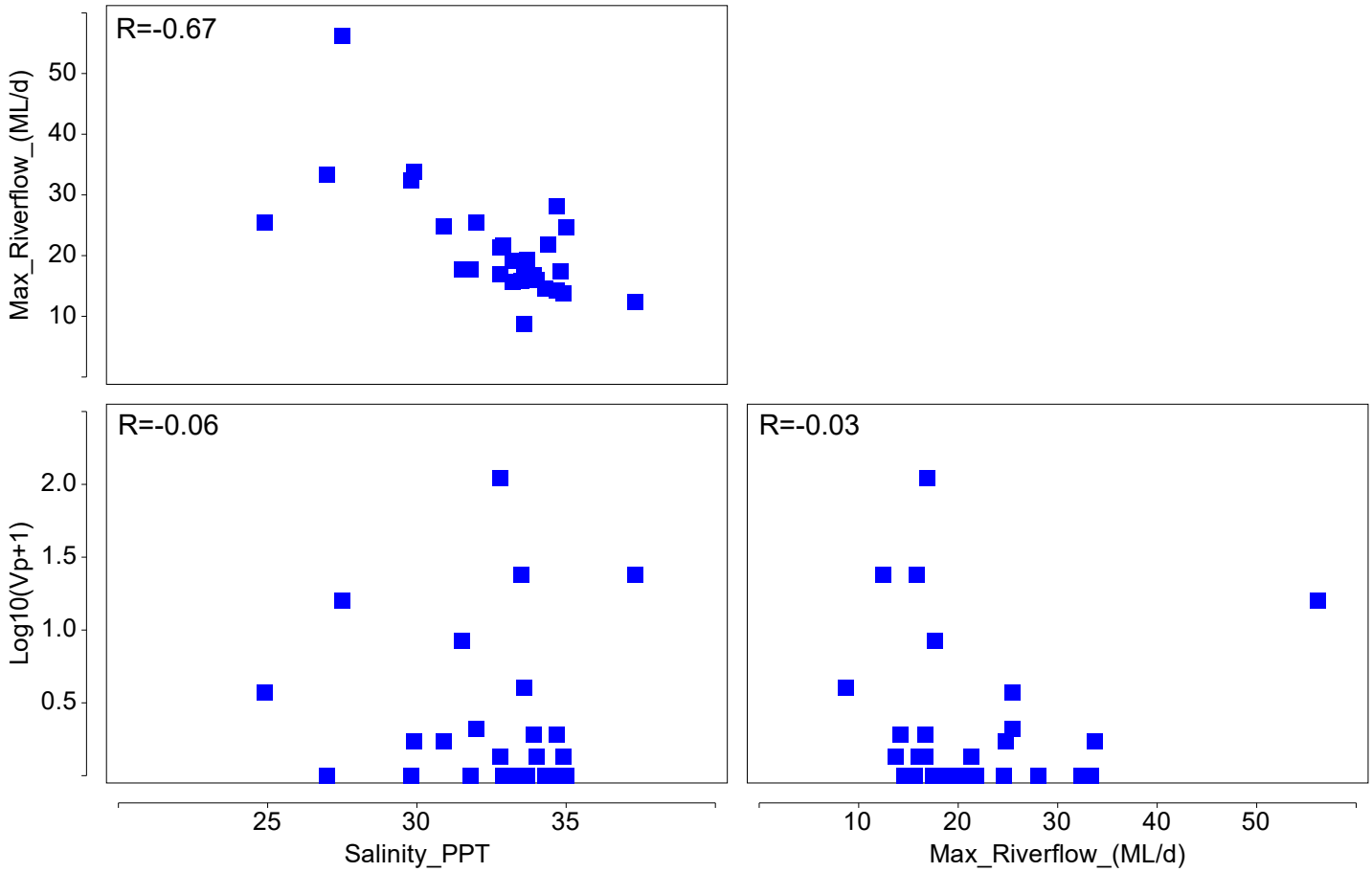

Figure 7: Draftsman's plots of salinity, river flow and *V. parahaemolyticus* variables.

Linear model generation

The marginal statistical tests in DISTLM showed significant relationships of sampling water temperature (Pseudo-F=8.5, P=0.006), 3-day average water temperature prior to sampling (Pseudo-F=23.7, P=0.001), minimum (Pseudo-F=15.1, P=0.002) and maximum (Pseudo-F=8.7, P=0.008) water temperature 3 days prior to sampling, to *V. parahaemolyticus* numbers, individually explaining 23%, 46%, 35% and 24% of the explained variability. In the most accurate predictive model selection 3-day average water temperature described 100% of the explained variability in the fitted model, 46% of the total variability in the data cloud (AICc=-53.24 R^2=0.46) (Figure 8).

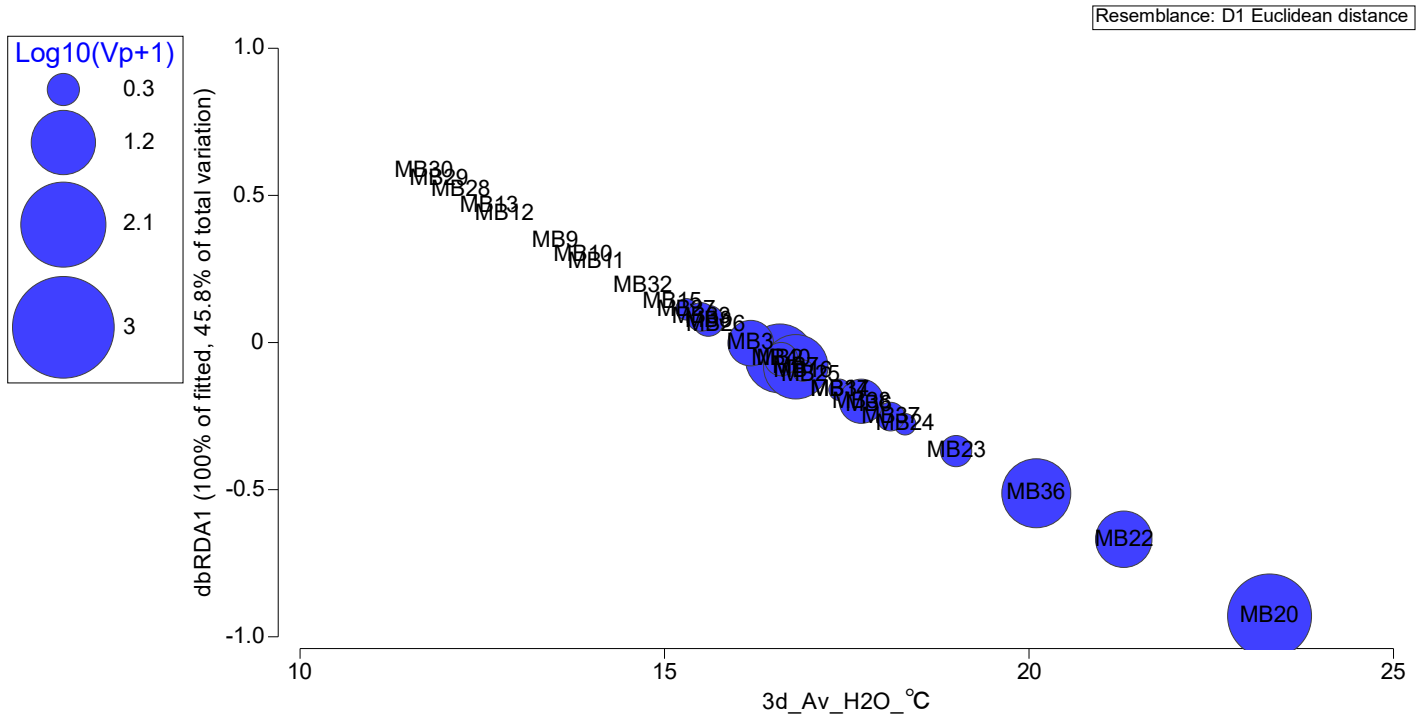

Figure 8: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with predictor variable 3-day average water temperature prior to sampling. Bubbles on plot indicate level of *V. parahaemolyticus* (Vp) detected in oysters from Moulting Bay.

The predictive model developed for *V. parahaemolyticus* in Moulting Bay was based on the 3-day average water temperature preceding shellfish harvest:

$$y = -1.7724 + 0.1302x$$

Where:
$y = \log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x = 3-day average water temperature (°C)
R^2 = 0.46.

Conclusions

- *V. parahaemolyticus* was detected in Moulting Bay in all sampling seasons between 2020 and 2022, although the prevalence was higher (67-89%) in the summer/autumn seasons as compared with the winter/spring seasons (17-20% prevalence).

- Levels of *V. parahaemolyticus* detected during the survey period were low; less than 110 MPN/g of oysters.

- *V. parahaemolyticus* carrying the *tdh* and *trh* genes, often associated with clinical strains, were only detected in the summer/autumn seasons with a prevalence of 13-25% for *tdh* and 0-13% for *trh*.

- *V. vulnificus* was only detected during the third summer/autumn sampling period and levels were <1 MPN/g in two oyster samples. The salinity of sampling water and average 3-day water temperature were 24.9 PSU and 17°C, and 32 PSU and 16°C.

- The various water temperature predictors were good indicators for V. parahaemolyticus risk. These predictors were correlated. *V. parahaemolyticus* was not detected when sampling water temperature was <14°C, when the 3-day average water temperature was <15°C or minimum water temperature 3-days prior to sampling was <13°C. The linear predictive model developed for *V. parahaemolyticus* risk in Moulting Bay was based on the 3-day average water temperature preceding shellfish harvest.

$$y = -1.7724 + 0.1302x$$

Where:

y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x= 3-day average water temperature prior to sampling (°C)
R^2 = 0.46

Appendix 3: *Vibrio* Survey Great Swanport (Oyster), 2020-2022

Background

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Great Swanport (Figure 1).

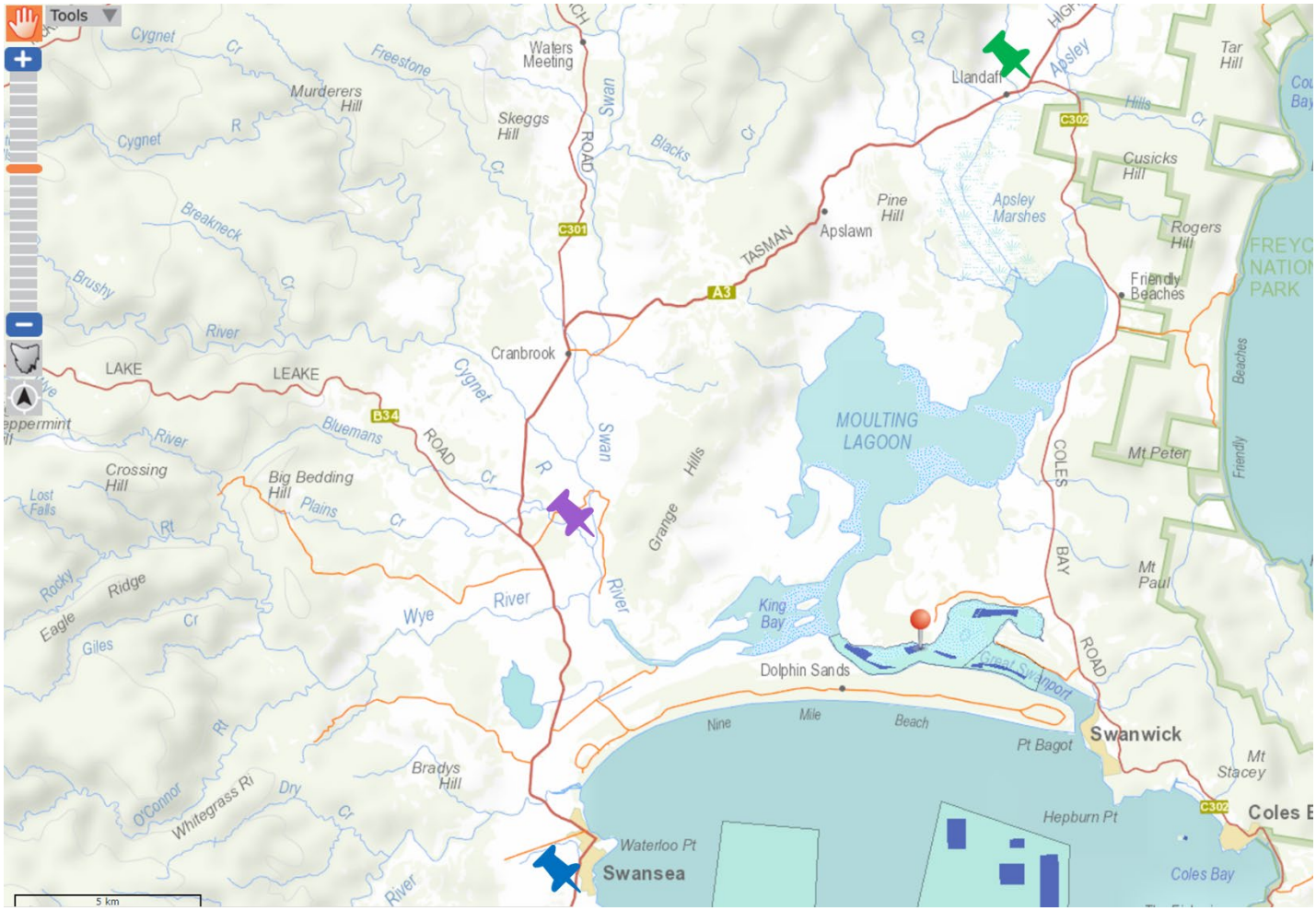

Figure 1: Great Swanport oyster harvest area. Orange pin indicates lease from which sampling occurred. The decimal degree (DD) coordinates of the oyster lease is -42.07799, 148.18444. Green pin indicates Apsley River flow station. Purple pin indicates Swan reiver flow station. Blue pin indicated BOM weather station at Swansea. Scale bar = 5 km.

Methodology

Summary of survey design

Oysters were sampled over three summer/autumn (November to April) and two winter/spring (May to October) periods from January 2020 to end of April 2022. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data collected: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data collected: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity, water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation.

Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station number 92148; Swansea - Francis Street). Maximum river flow on the day of sampling was also accessed from the Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable, for the river identified in the harvest area's sanitary survey. For Great Swanport these were both the Apsley River and Swan River. Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 92148; Swansea - Francis Street).

Statistical analysis

Statistical analysis of biological and environmental data was done in Primer 7 + PERMANOVA (Primer-e). Correlation among variables was investigated using draftsman's plots. *V. parahaemolyticus* or *V. vulnificus* (MPN/g) data were $\log_{10}(X+1)$ transformed and used to create a Euclidean distance resemblance matrix. The environmental data was also transformed if not normally distributed: rainfall and river flow data for Great Swanport was fourth root transformed. The DISTLM (distance-based linear model) routine was used to investigate the relationship between the *V. parahaemolyticus* or *V. vulnificus* Euclidean distance resemblance matrix and the environmental (temperature, salinity, rainfall and river flow) predictor variables. The step-wise "selection procedure" was used with the Akaike Information Criterion corrected for small sample sizes (AICc) "selection criterion" and 9999 permutations. AICc is a mathematical method for evaluating how well a model fits the data it was generated from. The output of this analysis gives a statistical marginal test for each individual predictor variable considered alone, and the best overall solution. The best linear model was visually represented using dbRDA (distance-based redundancy analysis). The formula for the resulting predictive model was generated using the regression function in excel.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Salinity and rainfall data during the survey period are shown in Figure 2A. The highest rainfall events were recorded in summer months and corresponded with increased river flow of the Swan and Apsley Rivers (Figure 2B). Figure 2C shows *V. parahaemolyticus* levels, sampling water temperature and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Temperature and *V. parahaemolyticus* numbers increased during summer/autumns months. Salinity in Great Swanport was variable (9-36 PSU) and drops in salinity corresponded to increased rainfall and river flow. The average salinity in summer/autumn was 29 PSU and in winter/spring it was 24 PSU.

Note that between May and September 2021 no oyster samples were collected. Hence no environmental data is presented during this period, and only one sample was collected during the second winter//spring sampling period.

During the survey period oyster and mussel samples were collected at the same timepoint in adjacent leases (oysters from -42.07799, 148.18444 and mussels from-42.07793, 148.18461). Environmental BOM data (air temperature and rainfall) and NRE river flow data are identical for the oyster and mussel samples from Great Swanport.

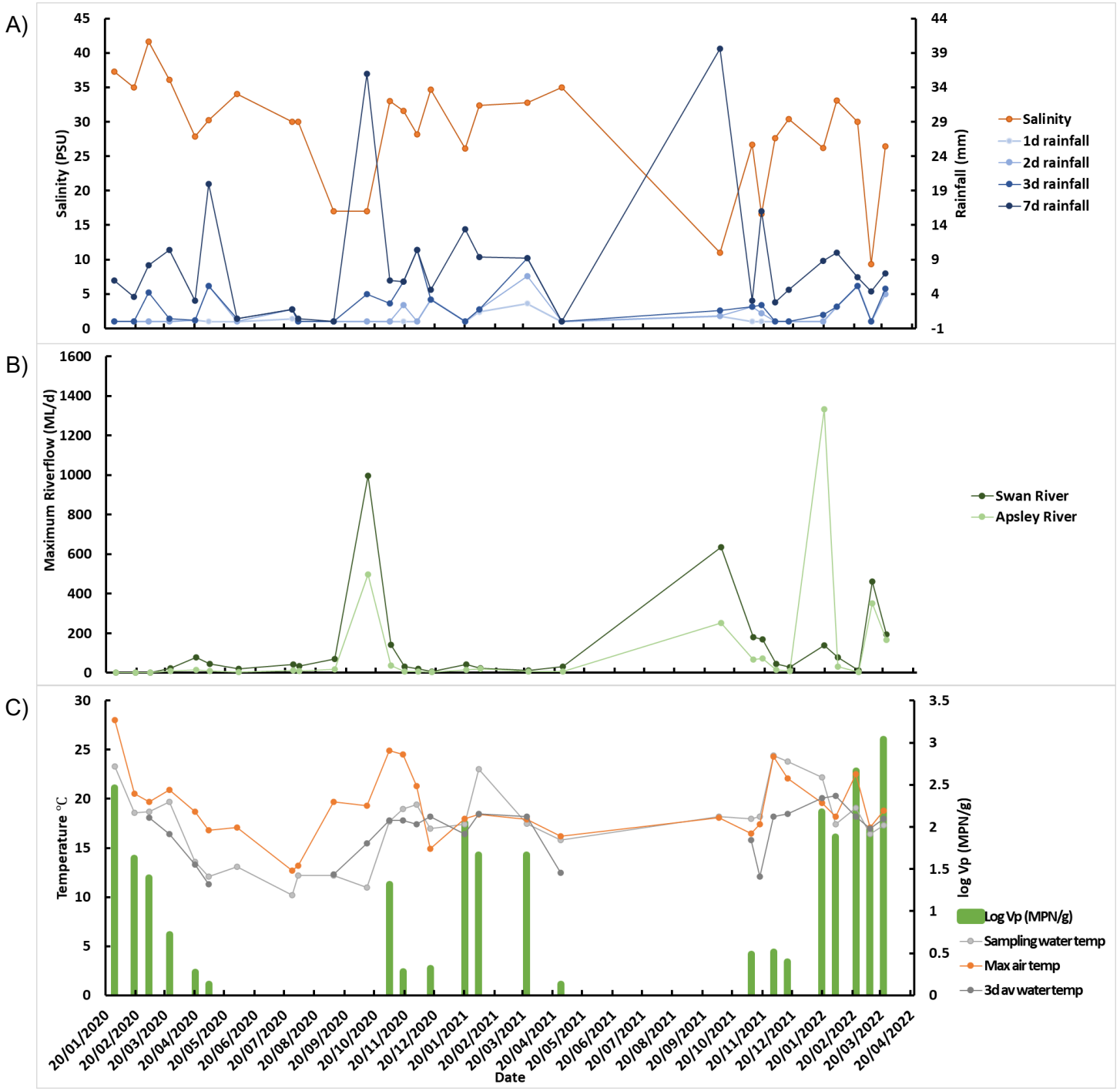

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Great Swanport (Oyster). 2A shows water salinity at sampling and rainfall at Swansea (1, 2, 3 and 7 days) prior to sampling. 2B shows maximum river flow of two rivers 24 hours prior to sampling. 2C shows levels of *V. parahaemolyticus* detected and are overlayed with sampling water temperature, three-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence for Great Swanport broken down to the 5 seasons sampled during the survey. A higher prevalence of *V. parahaemolyticus* were found during summer months (88-100%), although *V. parahaemolyticus* was detected all year round (noting that *V. parahaemolyticus* wasn't detected in the second winter/spring sampling period of 2021, however only one sample was taken during this period and is not representative of the entire season). *V. parahaemolyticus* strains containing the pathogenicity associated *tdh* and/or *trh* genes were detected with a low to moderate prevalence (11-33%) but only in the second and third

summer/autumn sampling periods. *V. vulnificus* was detected with a moderate to high prevalence (38-67%) during the second and third summer/autumn sampling periods.

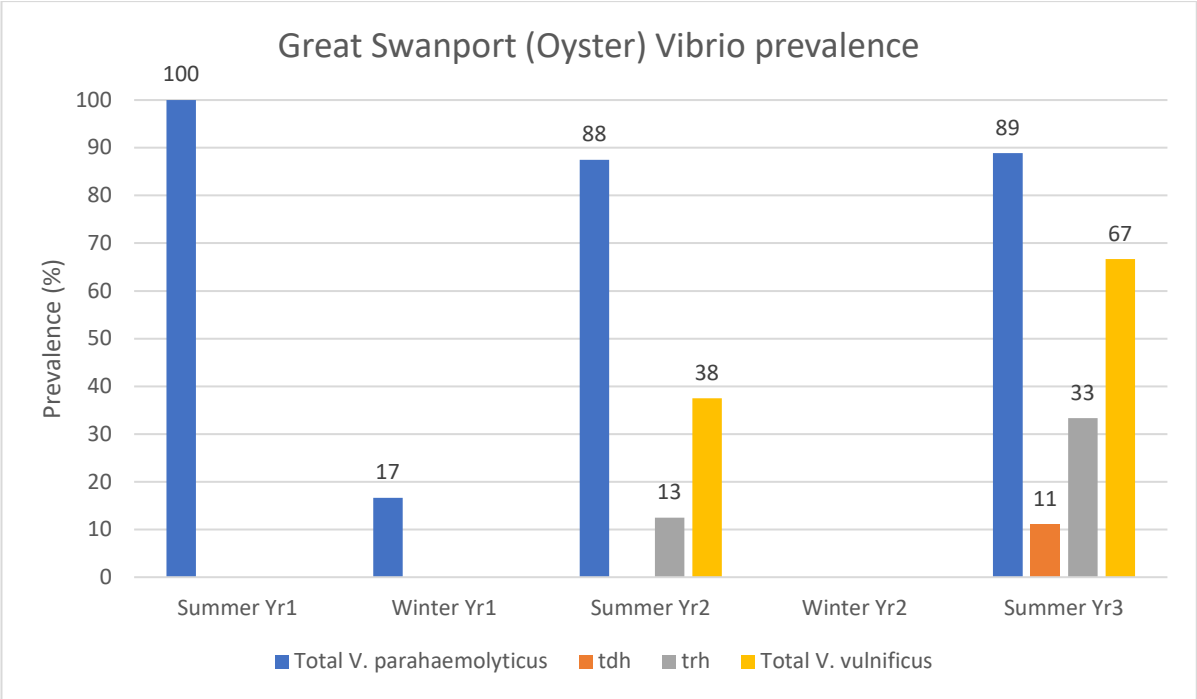

Figure 3: Seasonal *Vibrio* prevalence during the survey period.

V. parahaemolyticus was not detected in samples taken when sampling water temperature was <12°C, the 3-day average water temperature was <11°C or when minimum water temperature was <9°C (Figure 7). All samples were collected below water. No relationship could be deduced between tidal stage and *V. parahaemolyticus* detection (Figure 4).

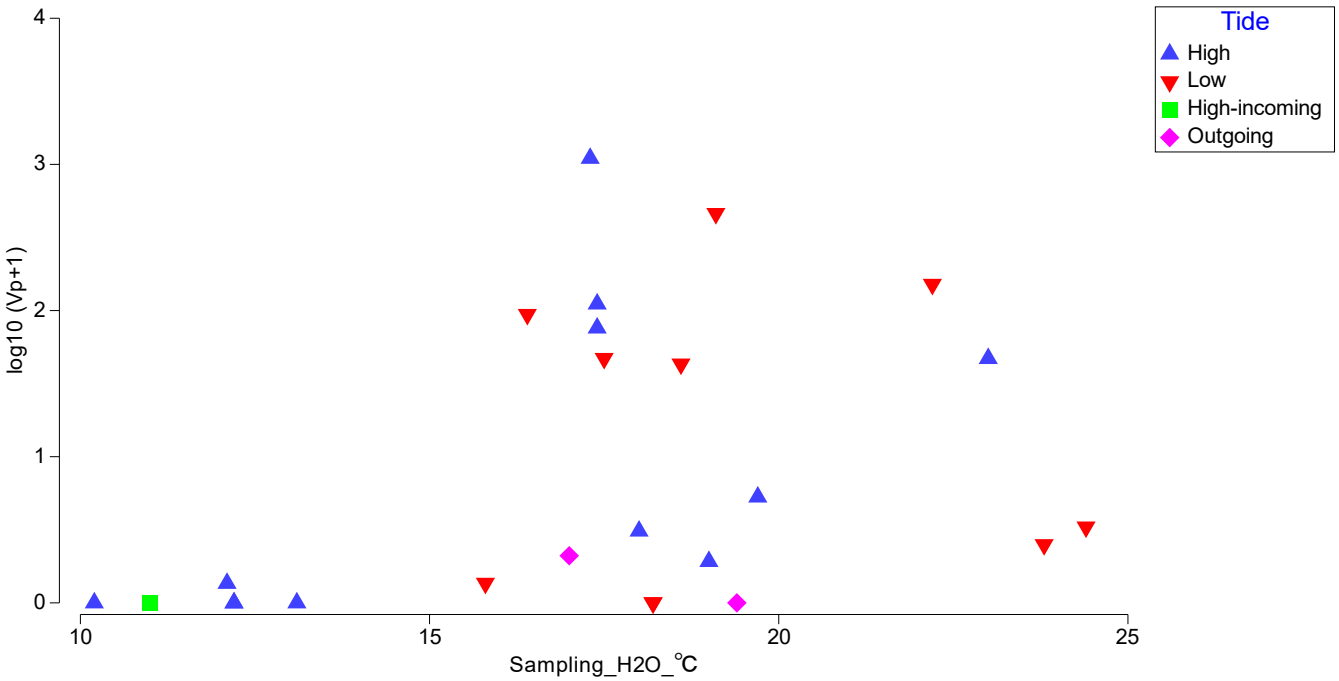

Figure 4: Scatter plot of *V. parahaemolyticus* versus sampling water temperature identified by tidal stage of collection.

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data during the survey aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of environmental conditions were recorded in the growing area over the period of the survey. High levels of up to 1,100 MPN/g *V. parahaemolyticus* were detected in non-abused oysters during the survey. *V. vulnificus* levels of ≤35 MPN/g were detected.

Table 1: Summary table of environmental parameters over the summer/autumn and winter/spring survey periods.

	Summer (n=18)					**Winter (n=7)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	15.8	24.4	19.1	2.7	18	10.2	18.2	12.7	2.6	7
Sampling air temp	16.0	26.4	20.7	2.9	11	23.2	23.2	23.2	NA	1
3-day min H_2O temp	6.0	16.5	12.6	2.8	17	7.0	10.5	9.0	1.8	3
3-day max H_2O temp	15.5	25.0	21.6	2.5	17	14.0	19.0	17.0	2.6	3
3-day Av H_2O temp	12.1	20.3	17.3	2.2	17	11.3	15.5	13.0	2.2	3
1-day max air temp	14.9	24.5	19.4	2.8	18	12.7	19.7	16.7	2.8	7
Salinity (PSU)										
Sampling H_2O	9.3	36.1	28.8	6.7	18	11.0	34.0	24.2	8.9	7
Rainfall (mm)										
1-day	0	5.2	1.0	1.7	18	0	0.8	0.2	0.3	7
2-days	0	6.6	1.6	2.0	18	0	5.2	1.1	2.0	7
3-days	0	10.4	2.7	3.2	18	0	5.2	1.9	2.0	7
7-days	0	16.0	7.2	4.1	18	0	39.6	14.0	17.7	7
River flow (ML/d)										
Swan River	0.02	460.4	82.9	113.7	18	21.0	995.2	263.4	391.5	7
Apsley River	0	1334	177.9	315.4	18	2.6	498.3	114.4	192.0	7

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods.

***Vibrio* enumeration (MPN/g)**						
Vibrio	**Summer (n=18)**			**Winter (n=7)**		
	Min	**Max**	**Positive (n=)**	**Min**	**Max**	**Positive (n=)**
Total *V. parahaemolyticus*	0	1100	16	0	0.36	1
tdh	0	2	1	0	0	0
trh	0	1.5	4	0	0	0
Total *V. vulnificus*	0	35	9	0	0	0

Statistical analysis

Temperature

The temperature predictor variables were correlated with one another (Figure 5). The various water temperature measures were more correlated with each other (R>0.45) than the air temperature and various water temperature measures (R<0.34). Maximum water temperature 3 day prior to shellfish sampling and the 3-day average water temperature were the most correlated followed by minimum and average water temperature 3 days prior to sampling. Both *V. parahaemolyticus* and *V. vulnificus* were most correlated with minimum water temperature 3 days prior to sampling (R= 0.63 and 0.41 respectively). *V. parahaemolyticus* and *V. vulnificus* numbers were also correlated (R=0.77).

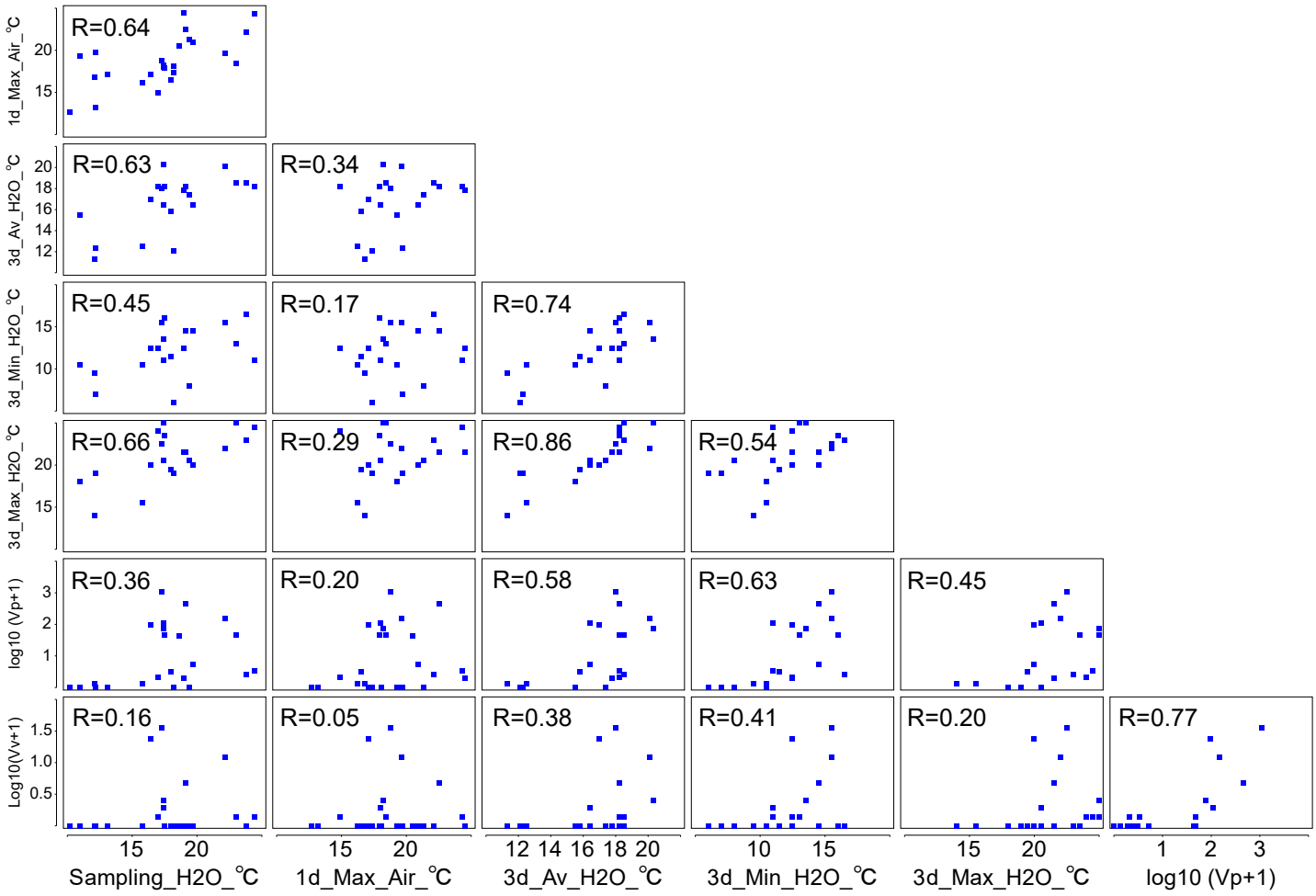

Figure 5: Draftsman's plots of temperature variables and *Vibrio*.

Rainfall

The rainfall measures were correlated with the highest correlation observed between one- and two-days rainfall and between two- and three-days rainfall (Figure 6). Poor correlation was observed between *Vibrio* numbers and rainfall, although one day rainfall was most correlated with both *V. parahaemolyticus* and *V. vulnificus* numbers.

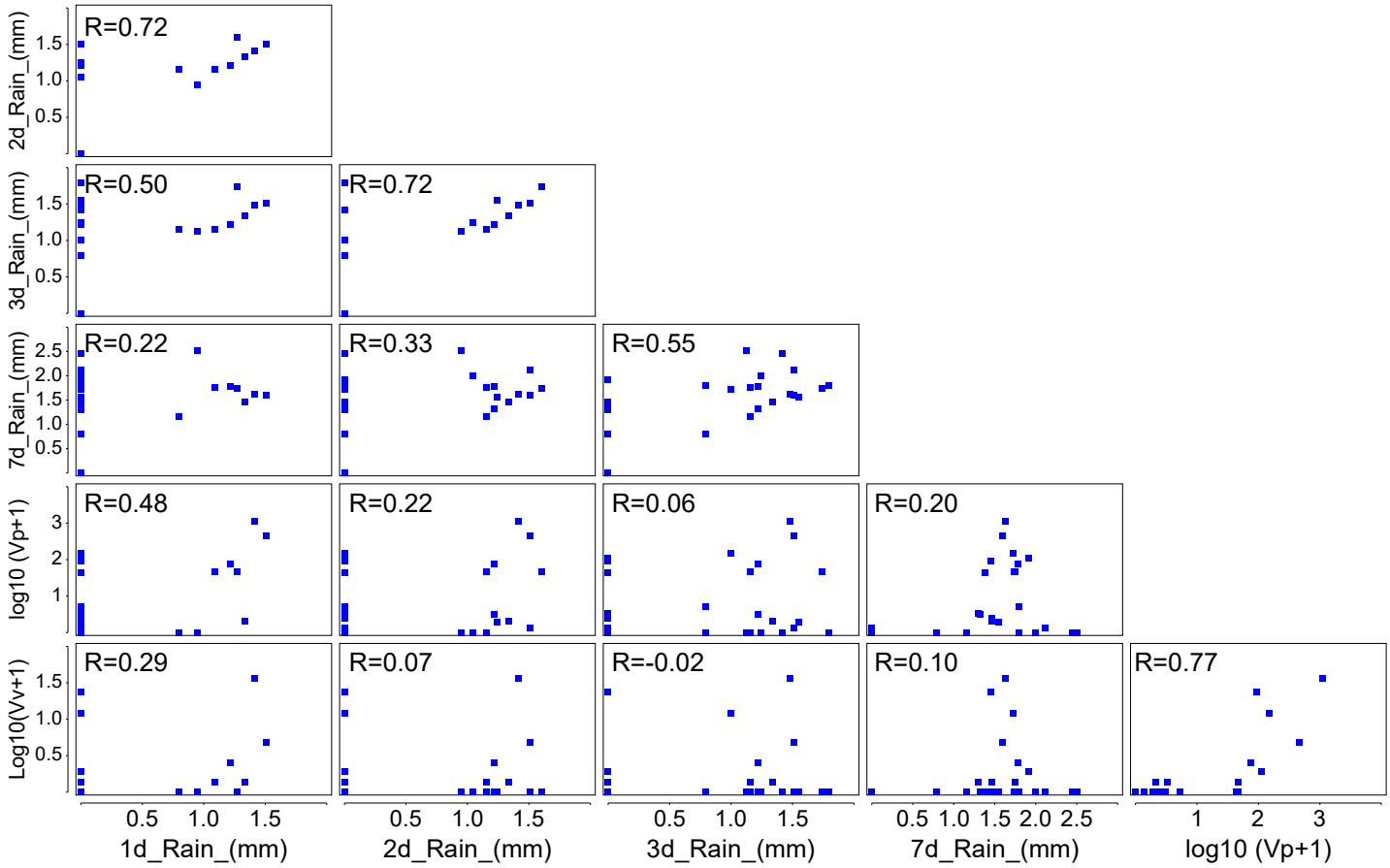

Figure 6: Draftsman's plots of rainfall variables and *Vibrio*.

Salinity and river flow

Maximum river flow and sampling water salinity were negatively correlated (Figure 7) with sampling water salinity most correlated to maximum river flow of the Swan River. *V. parahaemolyticus* numbers were not correlated with either sampling water salinity of maximum river flow. Maximum river flow of the Apsley and Swan Rivers were positively correlated (R=0.85). *V. parahaemolyticus* numbers were poorly correlated with salinity and maximum river flow. *V. vulnificus* numbers were somewhat positively correlated (R=0.56) with the maximal river flow of the Apsley River.

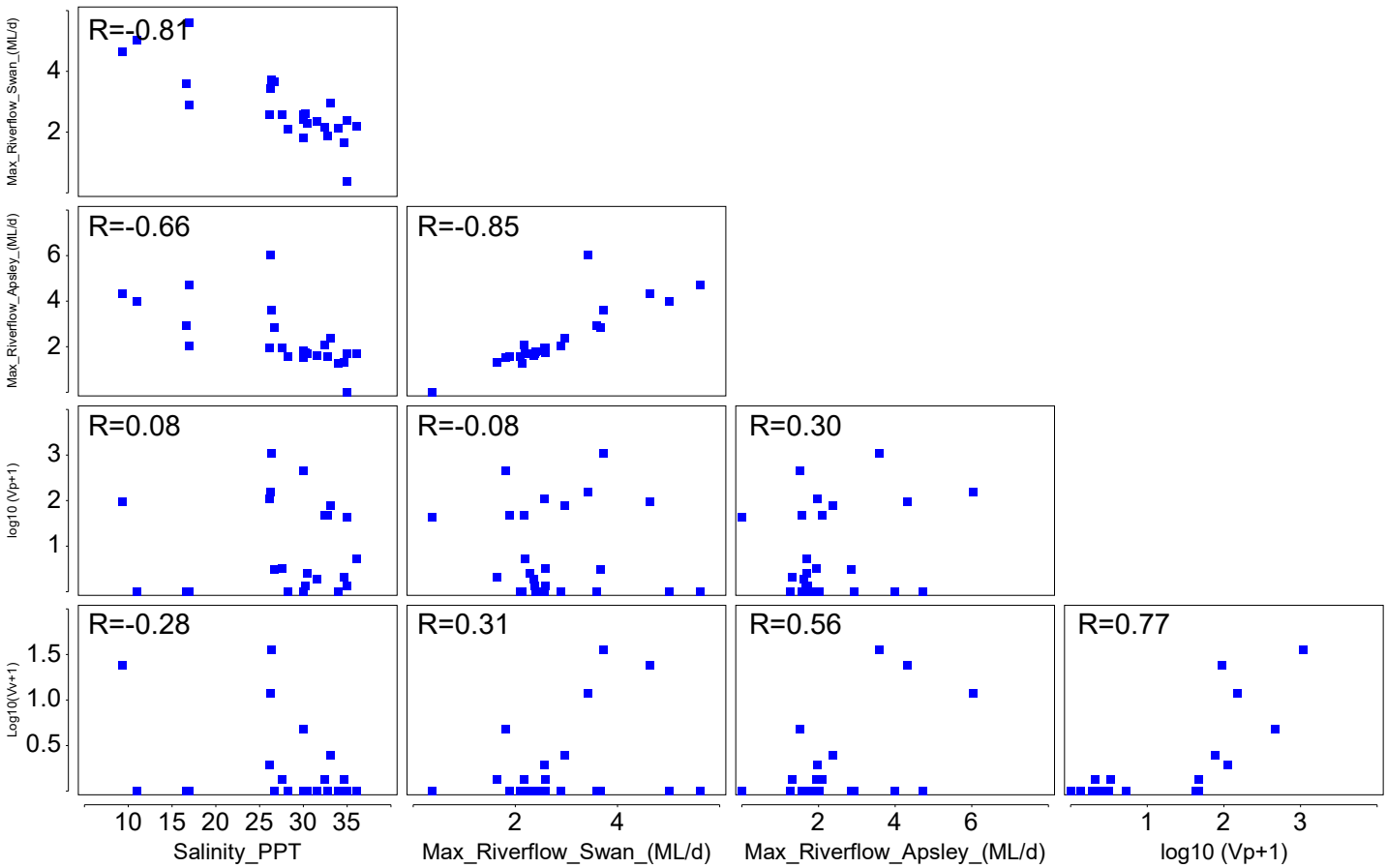

Figure 7: Draftsman's plots of salinity, river flow and *Vibrio* variables.

Linear model for V. parahaemolyticus

The marginal statistical tests in DISTLM showed significant relationships of 3-day average water temperature prior to sampling (Pseudo-F=8.9, P=0.007), minimum water temperature (Pseudo-F=12.1, P=0.003), maximum water temperature (Pseudo-F=4.5, P=0.050) and 1-d rainfall (Pseudo-F=10.0, P=0.006) to *V. parahaemolyticus* numbers, individually explaining 33%, 40%, 19% and 36% of the explained variability. However, after fitting minimum water temperature 3 days prior to sampling, salinity explained an additional 8% of the variability. Combined these two predictor variables described 100% of the explained variability in the fitted model, 48% of the total variability in the data cloud (AICc=-6.1592 R^2=0.48) (Figure 8).

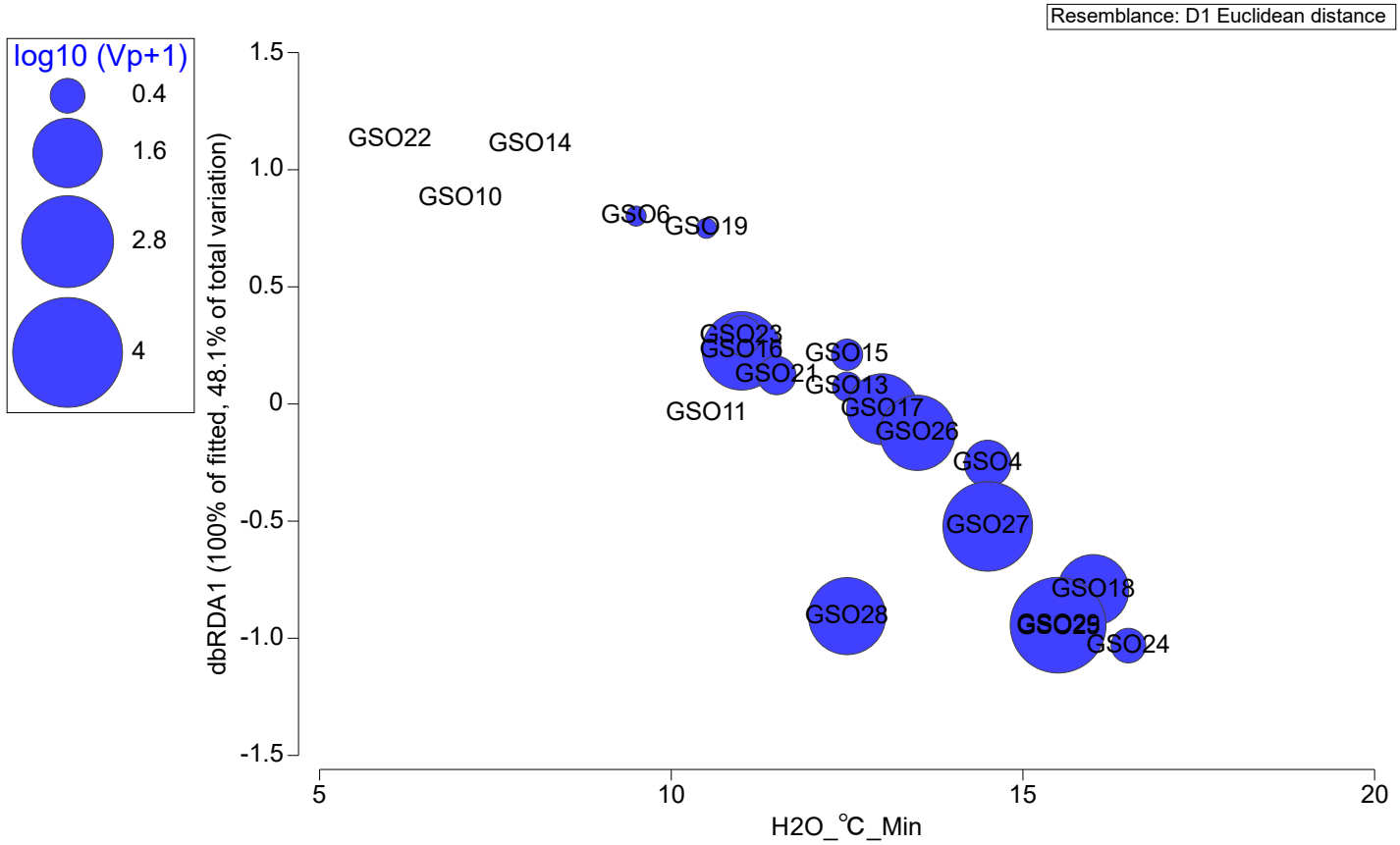

Figure 8: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with predictor variables minimum water temperature and salinity. Bubbles on plot indicate level of *V. parahaemolyticus* (Vp) detected in oysters sampled from Great Swanport.

The predictive model developed for *V. parahaemolyticus* in Great Swanport for oysters was based on minimum water temperature 3 days and sampling water salinity:

$$y = -0.9671 + 0.2632x_0 - 0.0440x_1$$

Where:

$y = \log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)

x_0 = minimum three-day water temperature prior to sampling (°C)

x_1 = sampling water salinity (PPT)

R^2=0.48

Linear model for *V. vulnificus*

The marginal statistical tests in DISTLM showed significant relationships of maximum river flow of the Apsley River (Pseudo-F=10.0, P=0.010) to *V. vulnificus* numbers, explaining 36% of the explained variability. However, after fitting maximal river flow of the Apsley, 1-d rainfall explained an additional 19% or the variability. Combined these two predictor variables described 100% of the explained variability in the fitted model, 55% of the total variability in the data cloud (AICc=-37.88 R^2=0.55) (Figure 9).

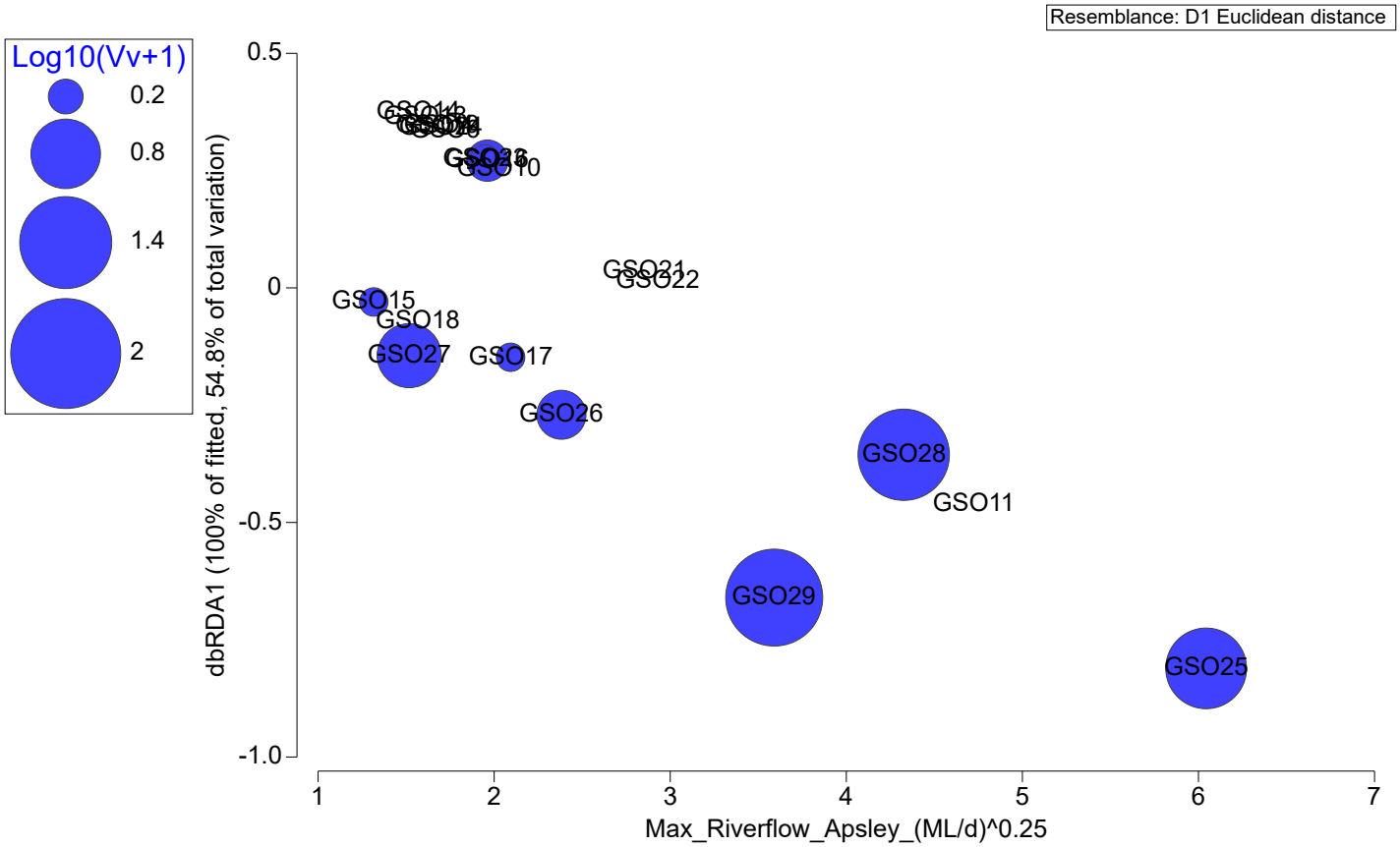

Figure 9: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with predictor variables maximum river flow of the Apsley River and one day rainfall. Bubbles on plot indicate level of *V. vulnificus* (Vv) detected in oysters sampled from Great Swanport.

The predictive model developed for *V. vulnificus* in Great Swanport for oysters was based on maximum river flow of the Apsley River and 1 day rainfall prior to sampling:

$$y = -0.5023 + 0.2651\sqrt[4]{x_0} + 0.3531\sqrt[4]{x_1}$$

Where:

$y = \log_{10}$ (*V. vulnificus* numbers + 1) (MPN/g)

$x_0 =$ maximum river flow of Apsley River (ML/d)

$x_1 =$ 1 day rainfall (mm)

R=0.55

Conclusions

- *V. parahaemolyticus* was detected all year round, with a higher prevalence in the summer/autumn (88-100%) as compared to 17% in the winter/spring sampling period. It should be noted that the second winter/spring sampling period in 2021 cannot be considered representative of that period as only one sample was collected.

- The levels of *V. parahaemolyticus* detected during the survey period were low to high (≤1,100 MPN/g in oysters).

- *V. parahaemolyticus* strains carrying the pathogenicity determinants *tdh* and/or *trh* were only detected in the summer/autumn sampling periods with a prevalence of 0-11% and 0-33%, respectively.

- Various water temperature predictors were good indicators of *V. parahaemolyticus* risk. *V. parahaemolyticus* was not detected in samples taken when sampling water temperature was <12°C, the 3-day average water temperature was <11°C or when minimum water temperature was <9°C.

  The linear predictive model developed for *V. parahaemolyticus* in Great Swanport for oysters was based on minimum water temperature 3 days prior to sampling and sampling water salinity:

$$y = -0.9671 + 0.2632x_0 - 0.0440x_1$$

  Where:
  $y = \log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
  $x_0 =$ minimum three-day water temperature prior to sampling (°C)
  $x_1 =$ sampling water salinity (PPT)
  R^2=0.48

- *V. vulnificus* was detected in two summer/autumn sampling periods with a prevalence of 38-67%. This prevalence was higher and more consistently observed across the summer/autumn sampling seasons than for any of the other harvest areas investigated for both mussels and oysters.

- Levels of *V. vulnificus* observed were ≤35 MPN/g in oysters. Salinity of sampling water associated with these positive samples ranged from 9-35 PSU and the sampling and average 3-day water temperatures ranged from 17-24°C and 16-20°C, respectively. Increased maximal river flow of the Apsley River was found to be significantly associated with increased *V. vulnificus* risk.

  The linear predictive model for *V. vulnificus* risk in Great Swanport for oysters was based on maximum river flow of the Apsley River and 1 day rainfall prior to sampling:

$$y = -0.5023 + 0.2651\sqrt[4]{x_0} + 0.3531\sqrt[4]{x_1}$$

  Where:
  $y = \log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
  $x_0 =$ maximum river flow of Apsley River (ML/d)
  $x_1 =$ 1 day rainfall (mm)
  R^2=0.55

- For both oysters and mussels in Great Swanport the predictors of *Vibrio* risk were similar; minimum water temperature 3 days prior to sampling and salinity for *V. parahaemolyticus* and maximum river flow of the Apsley River and 1 day rainfall for *V. vulnificus*.

Appendix 4: *Vibrio* Survey Great Swanport (Mussel), 2020-2022

Background

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Great Swanport (Figure 1).

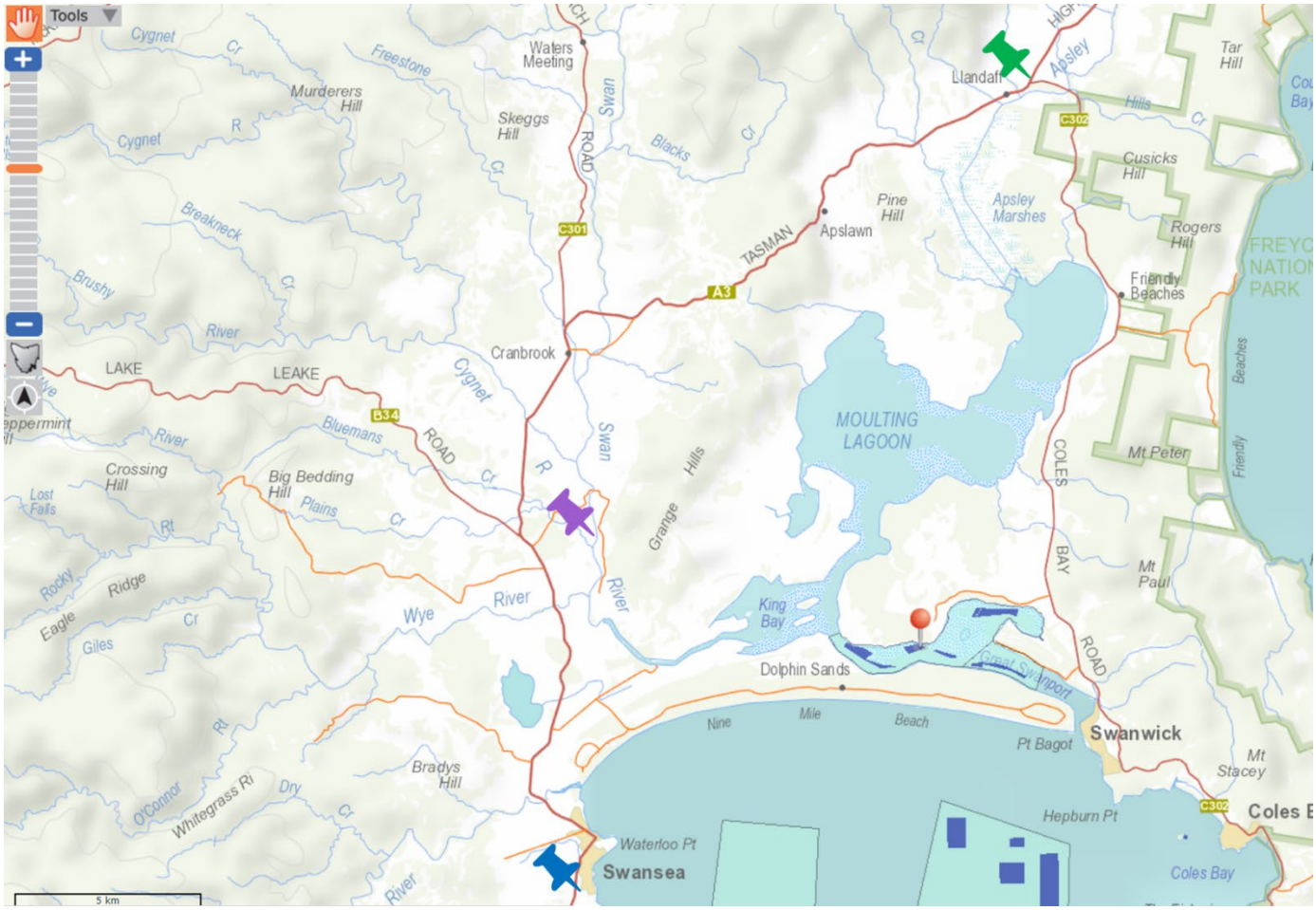

Figure 1: Great Swanport mussel harvest area. Orange pin indicates lease from which sampling occurred. The decimal degree (DD) coordinates of the mussel lease is -42.07793, 148.18461. Green pin indicates Apsley River flow station. Purple pin indicates Swan reiver flow station. Blue pin indicated BOM weather station at Swansea. Scale bar = 5 km.

Methodology

Summary of survey design

Mussels were sampled over three summer/autumn (November to April) and two winter/spring (May to October) periods from January 2020 to end of April 2022. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data collected: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data collected: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation. Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station number 92148; Swansea - Francis Street). Maximum river flow on the day of sampling was also accessed from the

Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable, for the river identified in the harvest area's sanitary survey. For Great Swanport these were both the Apsley River and Swan River. Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 92148; Swansea - Francis Street).

Statistical analysis

Statistical analysis of biological and environmental data was done in Primer 7 + PERMANOVA (Primer-e). Correlation among variables was investigated using draftsman's plots. *V. parahaemolyticus* or *V. vulnificus* (MPN/g) data were $\log_{10}(X+1)$ transformed and used to create a Euclidean distance resemblance matrix. The environmental data was also transformed if not normally distributed: rainfall and river flow data for Great Swanport was fourth root transformed. The DISTLM (distance-based linear model) routine was used to investigate the relationship between the *V. parahaemolyticus* or *V. vulnificus* Euclidean distance resemblance matrix and the environmental (temperature, salinity, rainfall and river flow) predictor variables. The step-wise "selection procedure" was used with the Akaike Information Criterion corrected for small sample sizes (AICc) "selection criterion" and 9999 permutations. AICc is a mathematical method for evaluating how well a model fits the data it was generated from. The output of this analysis gives a statistical marginal test for each individual predictor variable considered alone, and the best overall solution. The best linear model was visually represented using dbRDA (distance-based redundancy analysis). The formula for the resulting predictive model was generated using the regression function in excel.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Salinity and rainfall data during the survey period are shown in Figure 2A. The highest rainfall events were recorded in summer months and corresponded with increased river flow of the Swan and Apsley Rivers (Figure 2B). Figure 2C shows *V. parahaemolyticus* levels, sampling water temperature and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Temperature and *V. parahaemolyticus* numbers increased during summer/autumns months. Salinity in Great Swanport was variable (9-36 PSU) and drops in salinity corresponded to increased rainfall and river flow. The average salinity in summer/autumn was 29 PSU and in winter/spring it was 24 PSU.

Note that between May and September 2021 no mussel samples were collected. Hence no environmental data is presented during this period, and only one sample was collected during the second winter/spring sampling period.

During the survey period oyster and mussel samples were collected at the same timepoint in adjacent leases (oysters from 42.07799, 148.18444 and mussels from-42.07793, 148.18461). Environmental BOM data (air temperature and rainfall) and NRE river flow data are identical for the oyster and mussel samples from Great Swanport.

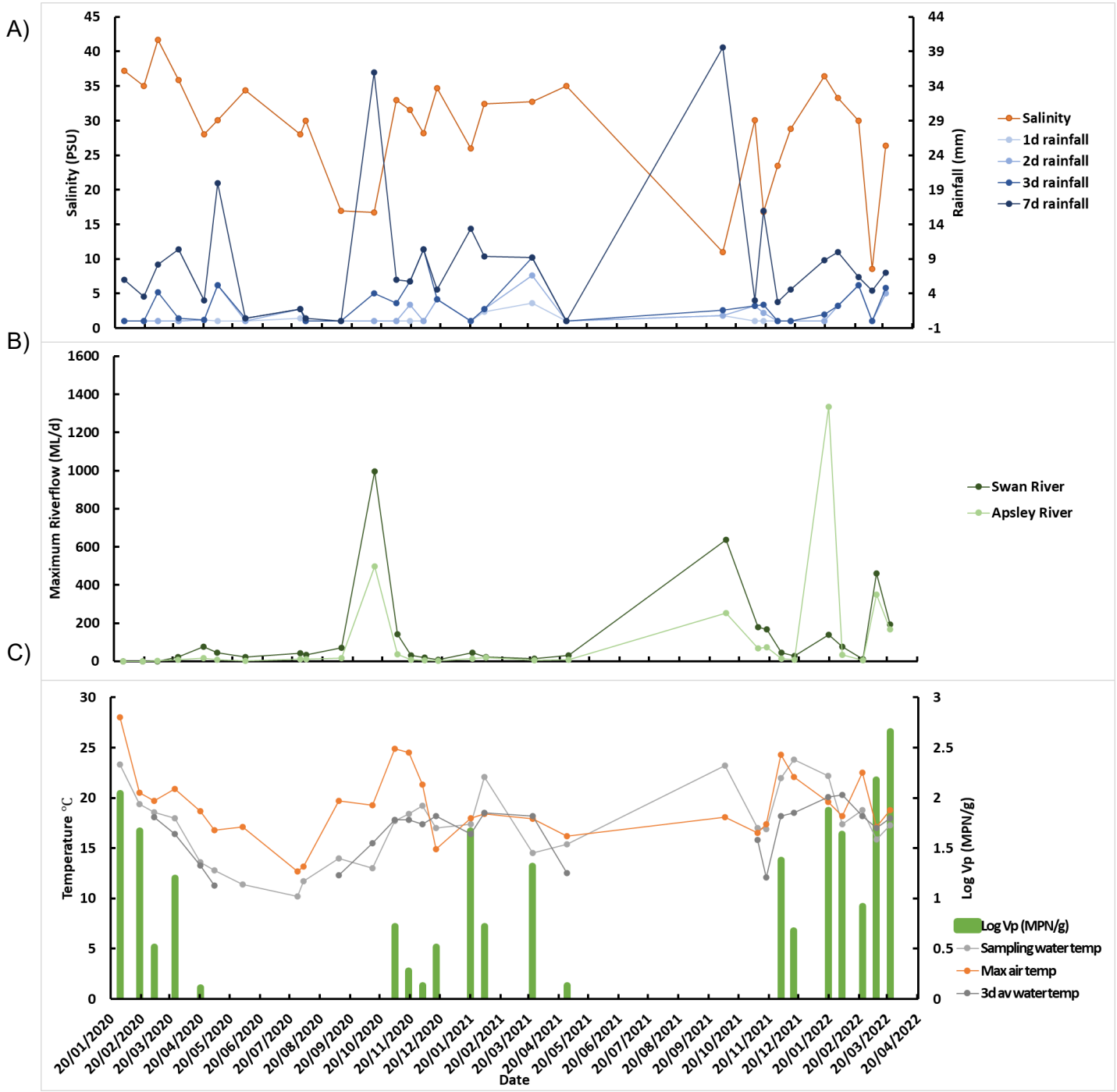

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Great Swanport (Mussel). 2A shows water salinity at sampling and rainfall at Swansea (1, 2, 3 and 7 days) prior to sampling. 2B shows maximum river flow of two rivers 24 hours prior of sampling. 2C shows levels of *V. parahaemolyticus* detected and are overlayed with sampling water temperature, three-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence for Great Swanport broken down to the 5 seasons sampled during the survey. A consistently high prevalence of *V. parahaemolyticus* was detected during the summer/autumn seasons during the survey period (78-100%). No *V. parahaemolyticus* was observed during the winter/spring periods, noting that only one sample was collected during the second winter/spring period. *V. parahaemolyticus* strains carrying the pathogenicity determinants *tdh* or *trh* were detected in the second and third summer/autumn

periods with a prevalence of 13-33%. *V. vulnificus* was detected in all the summer/autumn sampling period with a moderate to high prevalence of 20-67%.

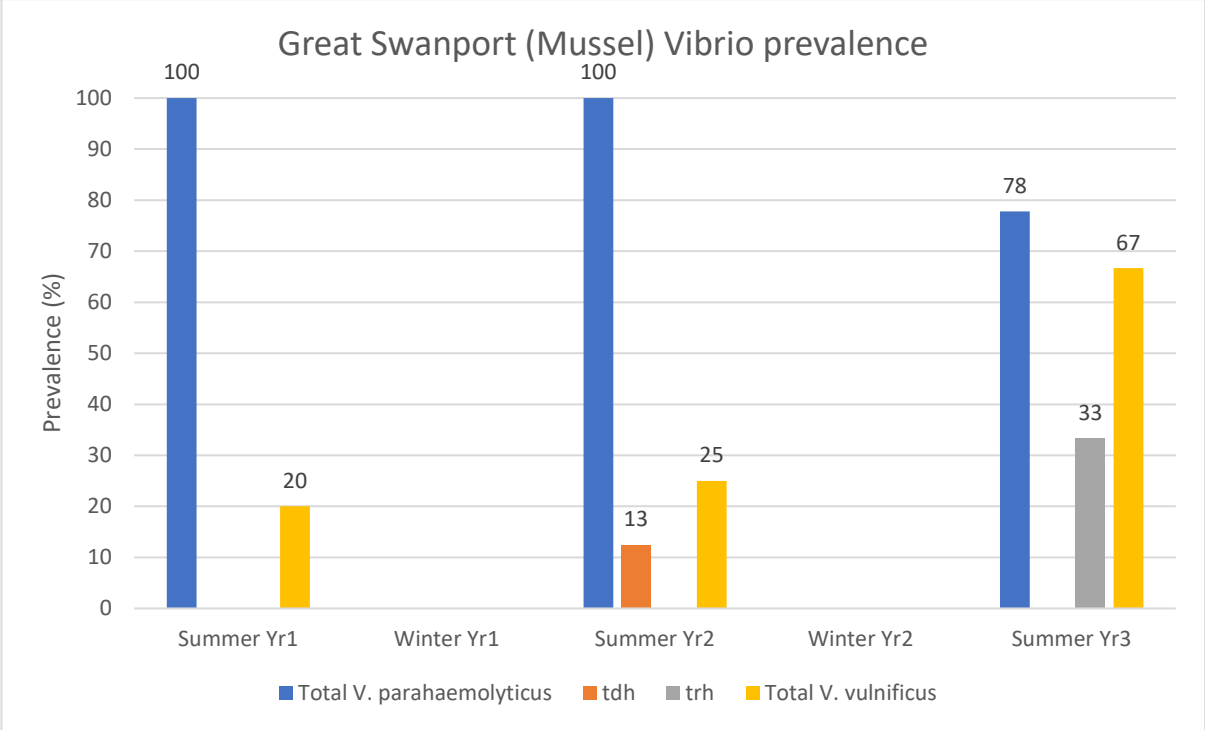

Figure 3: Seasonal *Vibrio* prevalence during the survey period.

V. parahaemolyticus was not detected in samples taken when sampling water temperature was <14°C, the 3-day average water temperature was <12°C or the minimum water temperature 3-days before sampling was <8°C. All samples but two samples were collected below water during the survey period (n=23/25). No relationship could be deduced between tidal stage and *V. parahaemolyticus* detection; positive samples were collected at both high and low tides (Figure 4).

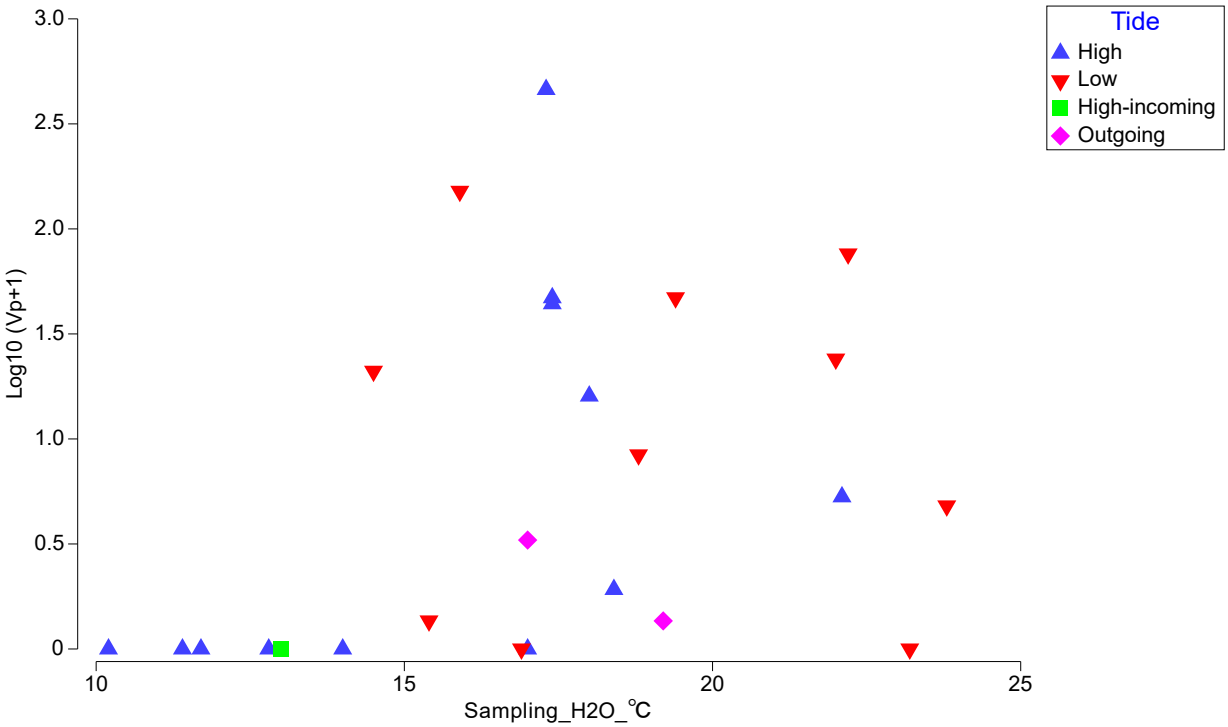

Figure 4: Scatter plot of *V. parahaemolyticus* versus sampling temperature identified by tidal stage of collection.

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data during the survey aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of environmental conditions were recorded in the growing area over the period of the survey. A maximum level of 460 MPN/g was detected for *V. parahaemolyticus* and *V. vulnificus* during the summer/autumn survey periods.

Table 1: Summary table of environmental parameters over the summer/autumn and winter/spring survey periods

	Summer (n=18)					**Winter (n=7)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	14.5	23.8	18.5	2.6	18	10.2	23.2	13.8	4.3	7
Sampling air temp	16.0	26.4	20.7	2.9	11	19.1	19.1	19.1	NA	1
3-day min H_2O temp	6.0	16.5	12.6	2.8	17	7.0	10.5	9.0	1.8	3
3-day max H_2O temp	15.5	25.0	21.6	2.5	17	14.0	19.0	17.0	2.6	3
3-day Av H_2O temp	12.1	20.3	17.3	2.2	17	11.3	15.5	13.0	2.2	3
1-day max air temp	14.9	24.5	19.4	2.8	18	12.7	19.7	16.7	2.8	7
Salinity (PSU)										
Sampling H_2O	8.5	36.4	29.2	7.2	18	11.0	34.4	23.9	8.8	7
Rainfall (mm)										
1-day	0	5.2	1.0	1.7	18	0	0.8	0.2	0.3	7
2-days	0	6.6	1.6	2.0	18	0	5.2	1.1	1.9	7
3-days	0	10.4	2.7	3.2	18	0	5.2	1.9	2.0	7
7-days	0	16.0	7.2	4.1	18	0	39.6	14.0	17.7	7
River flow (ML/d)										
Swan River	0.02	460.4	82.9	113.7	18	21.0	995.2	263.4	391.5	7
Apsley River	0	1334	117.9	315.4	18	2.6	498.3	114.4	192.0	7

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods

***Vibrio* enumeration (MPN/g)**						
Vibrio	**Summer (n=18)**			**Winter (n=7)**		
	Min	**Max**	**Positive (n=)**	**Min**	**Max**	**Positive (n=)**
Total *V. parahaemolyticus*	0	460	16	0	0	0
tdh	0	0.36	1	0	0	0
trh	0	2.3	3	0	0	0
Total *V. vulnificus*	0	460	8	0	0	0

Statistical analysis

Temperature

The temperature predictor variables were somewhat correlated. Maximum daily air temperature was the least correlated with any of the other water temperature predictors, other than sampling water temperature (Figure 5). The 3-day average water temperature and minimum and maximum water temperature prior to sampling were the most correlated (R>0.74). *V. parahaemolyticus* and *V. vulnificus* were most correlated with the minimum water temperature 3 days prior to sampling (R=0.62 and 0.34 respectively). *V. parahaemolyticus* and *V. vulnificus* numbers were also correlated (R=0.72).

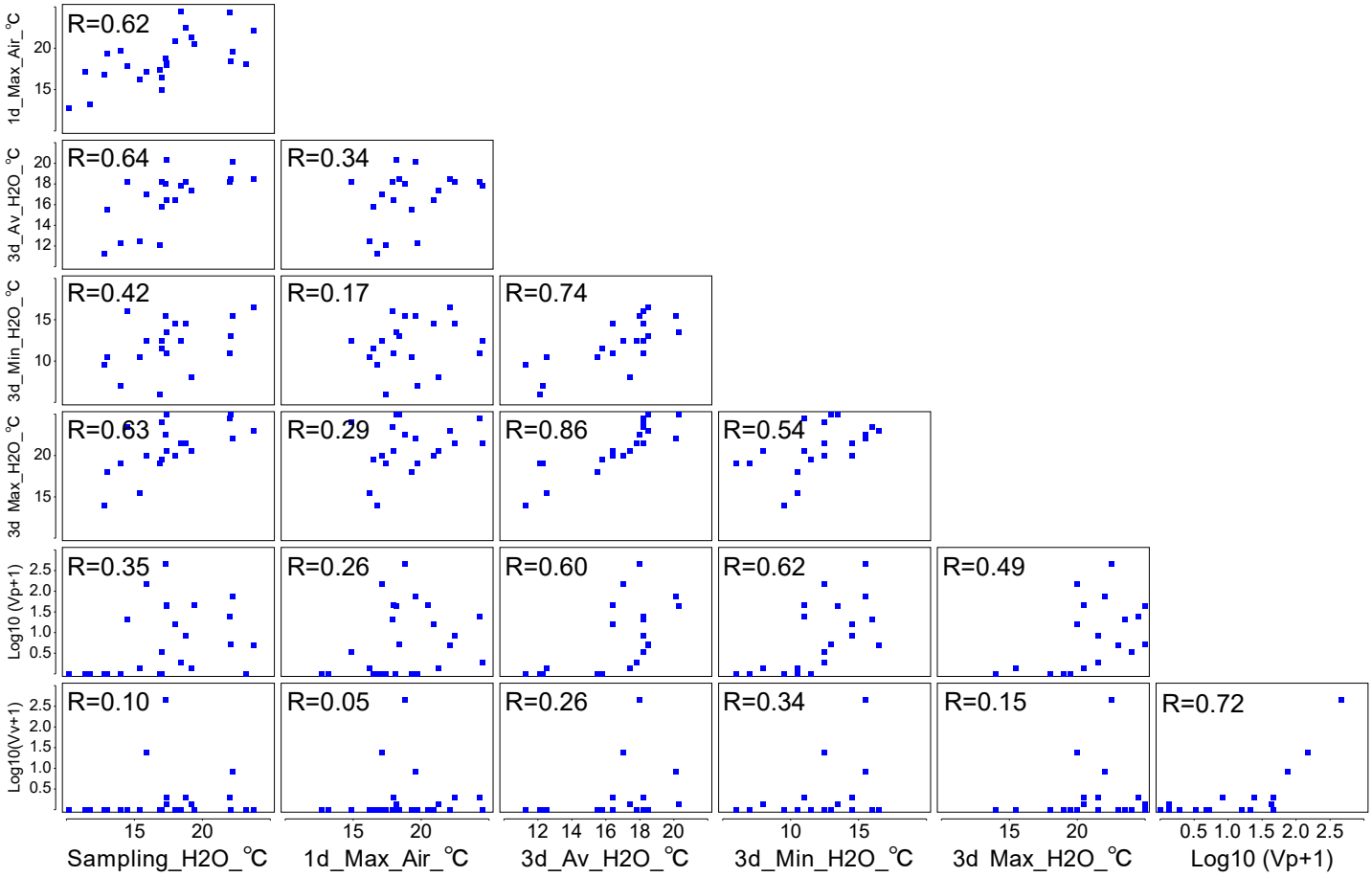

Figure 5: Draftsman's plots of temperature variables and *Vibrio*.

Rainfall

The rainfall measures were correlated with one another with the highest correlation observed between one- and two-days rainfall and between two- and three-days rainfall (Figure 6). Poor correlation was observed between *Vibrio* numbers (*V. parahaemolyticus* and *V. vulnificus*) and rainfall.

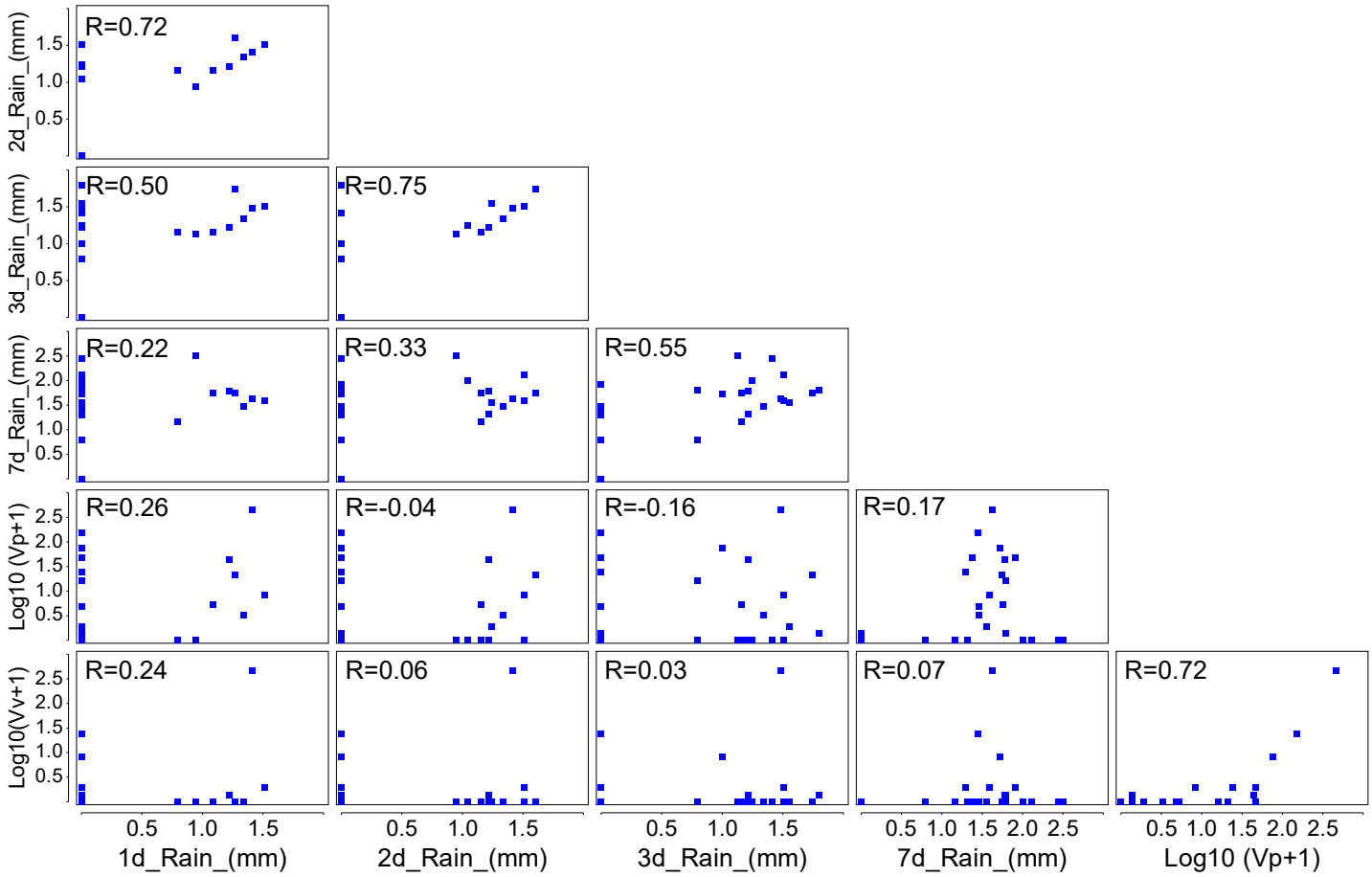

Figure 6: Draftsman's plots of rainfall variables and *Vibrio*.

Salinity and river flow

Maximum river flow and sampling water salinity were negatively correlated for both the Apsley and Swan rivers; the correlation was greatest for the Swan River (Figure 7). River flow was highly positively correlated (R=0.85) between the Swan and the Apsley rivers. *Vibrio* numbers were not correlated with sampling water salinity, although *V. vulnificus* was somewhat correlated with maximum river flow of the Apsley River.

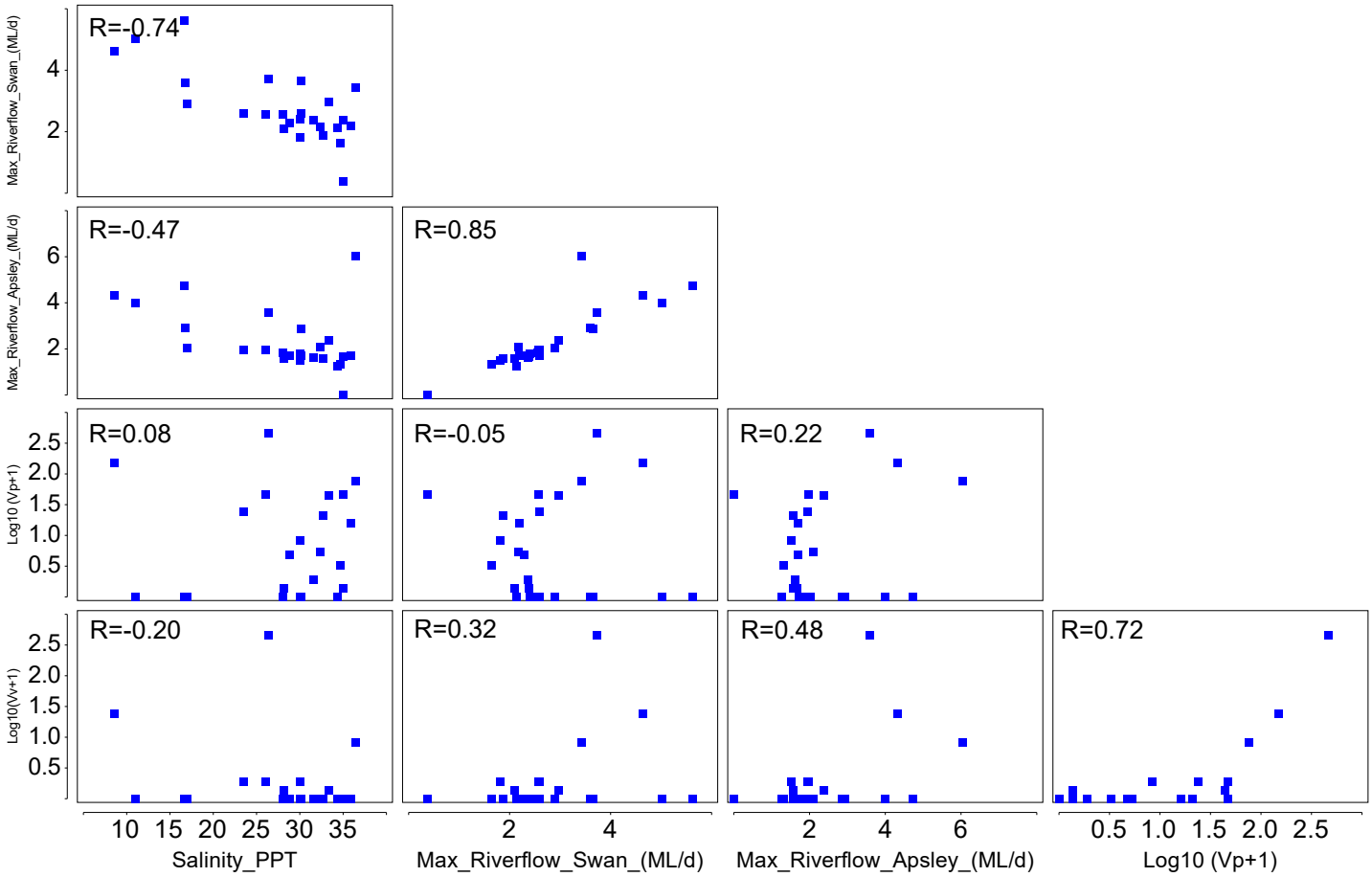

Figure 7: Draftsman's plots of salinity, river flow and *Vibrio* variables.

Linear model generation for *V. parahaemolyticus*

The marginal statistical tests in DISTLM showed significant relationships of 3-day average water temperature prior to sampling (Pseudo-F=10.3, P=0.005) and minimum (Pseudo-F=11.4, P=0.004) and maximum (Pseudo-F=5.8, P=0.020) water temperature 3 days prior to sampling, to *V. parahaemolyticus* numbers, individually explaining 36%, 39% and 24% of the explained variability. However, after fitting minimum water temperature 3 days prior to sampling, salinity explained an additional 12% or the variability. These two predictor variables described 100% of the explained variability in the fitted model, 50% of the total variability in the data cloud (AICc=-14.867 R^2=0.50) (Figure 8).

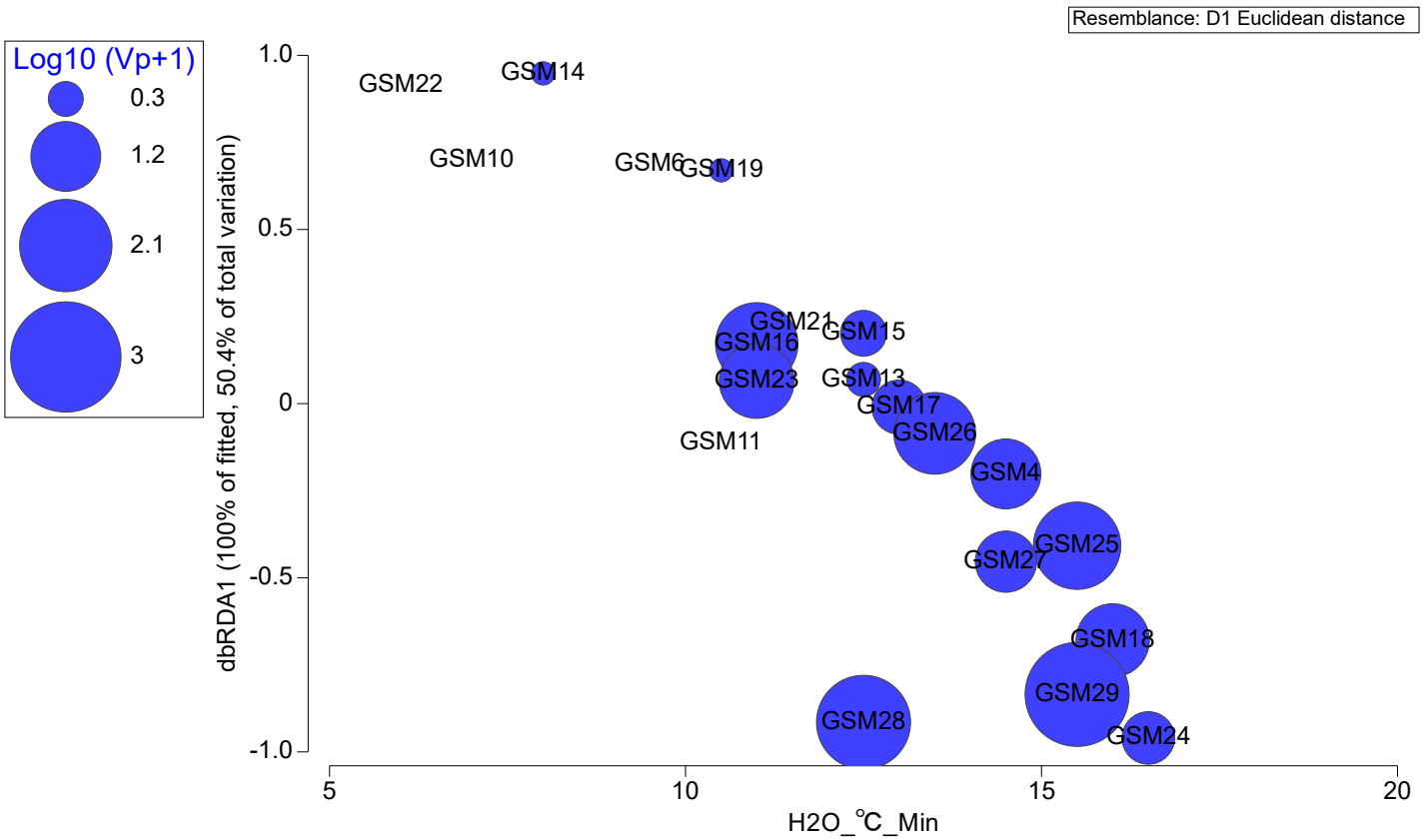

Figure 8: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with predictor variables minimum water temperature and salinity. Bubbles on plot indicate level of *V. parahaemolyticus* (Vp) detected in mussels sampled from Great Swanport.

The predictive model developed for *V. parahaemolyticus* in Great Swanport for mussels was based on the minimum water temperature 3 days prior to harvest and harvest water salinity:

$$y = -0.6970 + 0.2275x_0 - 0.0427x_1$$

Where:

y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)

x_0 = minimum water temperature 3 days prior to sampling (°C)

x_1 = sampling water salinity (PPT)

R^2 = 0.50

Linear model generation for *V. vulnificus*

The marginal statistical tests in DISTLM showed a significant relationship between maximum river flow of the Apsley River (Pseudo-F=6.4, P=0.039) and *V. vulnificus* numbers, explaining 26% of the variability. However, after fitting maximal river flow of the Apsley, 1-d rainfall explained an additional 13% or the variability. Combined these two predictor variables described 100% of the explained variability in the fitted model, 40% of the total variability in the data cloud (AICc=-20.237 R^2=0.40) (Figure 9).

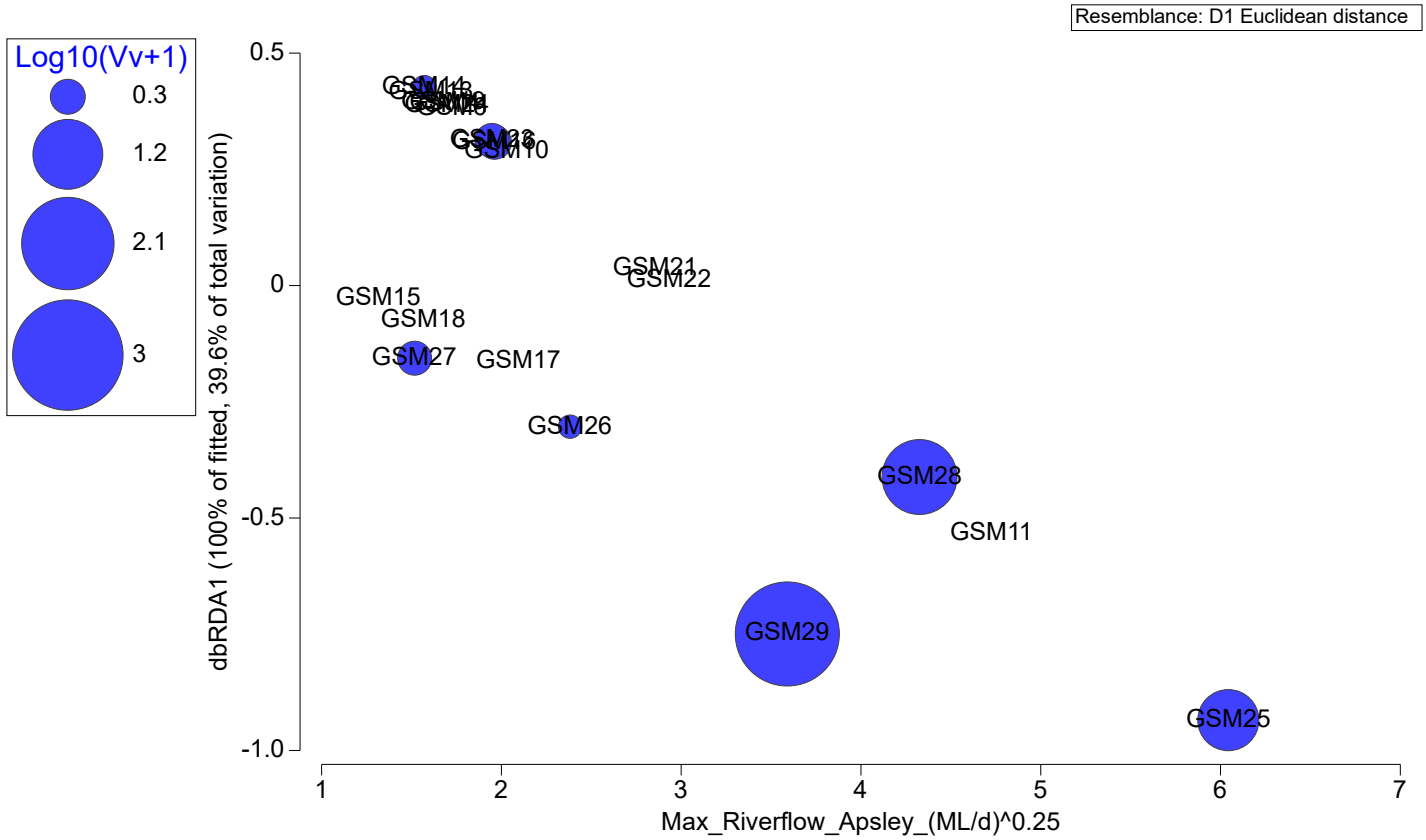

Figure 9: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with predictor variables maximum river flow of the Apsley River and one day rainfall. Bubbles on plot indicate level of *V. vulnificus* (Vv) detected in mussels sampled from Great Swanport.

The predictive model developed for *V. vulnificus* in Great Swanport for mussels was based on the maximum river flow of the Apsley River and 1 day rainfall prior to sampling:

$$y = -0.6013 + 0.30446\sqrt[4]{x_0} + 0.39704\sqrt[4]{x_1}$$

Where:

y = $\log_{10}$ (*V. vulnificus* numbers + 1) (MPN/g)

x_0 = maximum river flow of the Apsley River (ML/d)

x_1 = 1 day rainfall (mm)

R^2 = 0.40

Conclusions

- *V. parahaemolyticus* was only detected in the summer/autumn sampling periods, with a high prevalence of 78-100%.
- No *V. parahaemolyticus* was detected in the winter/spring sampling periods. Although, it should be noted that the second winter/spring sampling period in 2021 cannot be considered representative of that period as only one sample was collected.
- The levels of *V. parahaemolyticus* detected during the survey period were low to moderate (≤460 MPN/g in mussels).
- *V. parahaemolyticus* strains carrying the pathogenicity determinants *tdh* or *trh* were only detected in the summer/autumn sampling periods with a prevalence of 0-13% and 0-33%, respectively.
- The average, minimum and maximum water temperature 3 days preceding sampling were good indicators of *V. parahaemolyticus* risk. *V. parahaemolyticus* was not detected in samples taken when sampling water temperature was <14°C, the 3-day average water temperature was <12°C or the minimum water temperature 3-days before sampling was <8°C. These predictors were correlated. The predictive model for *V. parahaemolyticus* incorporated both the minimum water temperature 3 days prior to sampling and sampling water salinity.

$$y = -0.6970 + 0.2275x_0 - 0.0427x_1$$

Where:
y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = minimum water temperature 3 days prior to sampling (°C)
x_1 = sampling water salinity (PPT)
R^2 = 0.50

- *V. vulnificus* was detected in all three summer/autumn sampling periods with a prevalence of 20-67%. This prevalence was higher and more consistently observed across the summer/autumn sampling seasons than for any of the other harvest areas investigated for both oysters and mussels.
- Levels of *V. vulnificus* observed were ≤460 MPN/g in mussels. Salinity of sampling water associated with these positive samples ranged from 9-37 PSU and the average 3-day water temperature ranged from 16-20°C.
- Increased maximal river flow of the Apsley River was found to be significantly associated with increased *V. vulnificus* risk in Great Swanport. The predictive model for *V. vulnificus* incorporated both maximum river flow of the Apsley River and one day rainfall data.

$$y = -0.6013 + 0.30446\sqrt[4]{x_0} + 0.39704\sqrt[4]{x_1}$$

Where:
y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = maximum river flow of the Apsley River (ML/d)
x_1 = 1 day rainfall (mm)
R^2 = 0.40

- For both mussels and oysters in Great Swanport the predictors of *Vibrio* risk were similar; minimum water temperature 3 days prior to sampling and salinity for *V. parahaemolyticus* and maximum river flow of the Apsley River and 1 day rainfall for *V. vulnificus*.

Appendix 5: *Vibrio* Survey Great Oyster Bay, 2020

Background

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Great Oyster Bay (Figure 1).

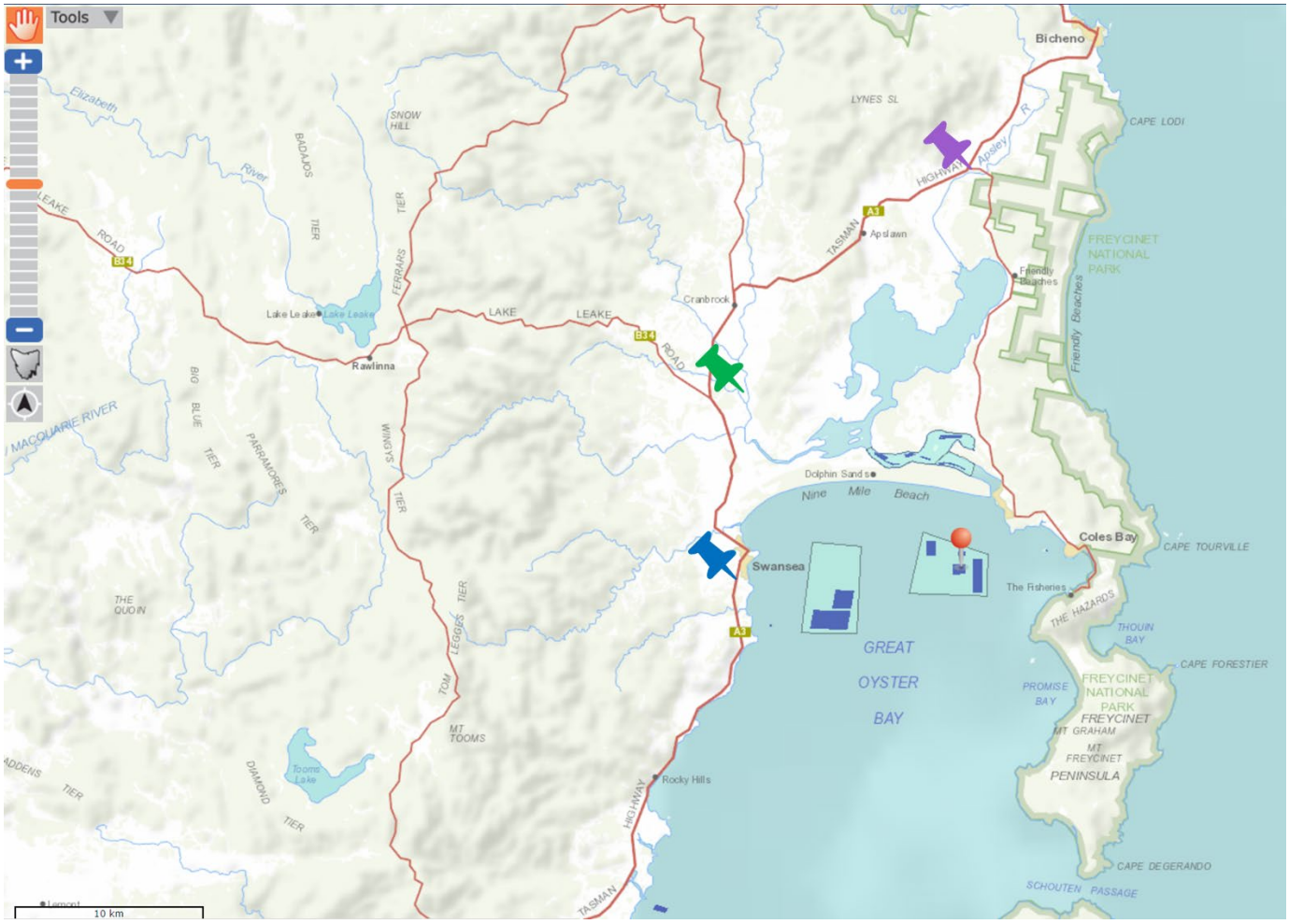

Figure 1: Great Oyster Bay harvest area. Orange pin indicates lease from which sampling occurred. Green pin indicates Swan River flow station. Purple pin indicates Apsley River flow station. Blue pin indicates BOM weather station at Swansea Francis Street. Scale bar = 10 km.

Methodology

Summary of survey design

Oysters were sampled over two partial summer/autumn (November to April) and one winter/spring (May to October) periods from January to December 2020. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data collected: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data collected: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity, water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation. Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station

number 92148; Swansea Francis Street). Maximum river flow on the day of sampling was also accessed from the Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable, for the river identified in the harvest areas' sanitary survey. For the Great Oyster Bay harvest area these were the Swan and Apsley Rivers. Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 92148; Swansea Francis Street).

Statistical analysis

Due to the limited number of samples submitted over the survey period (n=14) of which 10 were not temperature abused, and even less environmental predictor data submitted around harvest water temperature, no statistical analysis could be done for Great Oyster Bay. Samples were only submitted during 2020. The results have been summarised below.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Only 14 samples were collected from Great Oyster Bay over the first two summer/autumn and first winter/spring sampling periods. Difficult in sampling this harvest area was identified early in the survey due to unfavourable environmental conditions. Of the samples taken four were considered temperature abused and discounted from further statistical analysis. The limited data presented makes statistical analysis difficult.

Salinity and rainfall data during the survey period are shown in Figure 2A. The highest rainfall events were recorded in autumn and spring of 2020, which corresponded with increased flow of the Swan and Apsley Rivers (Figure 2B). Figure 2C shows *V. parahaemolyticus* levels, sampling water temperature and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Temperature and *V. parahaemolyticus* (n=3) numbers increased during summer/autumns months. Salinity in Great Oyster Bay was constant (35-36 PSU).

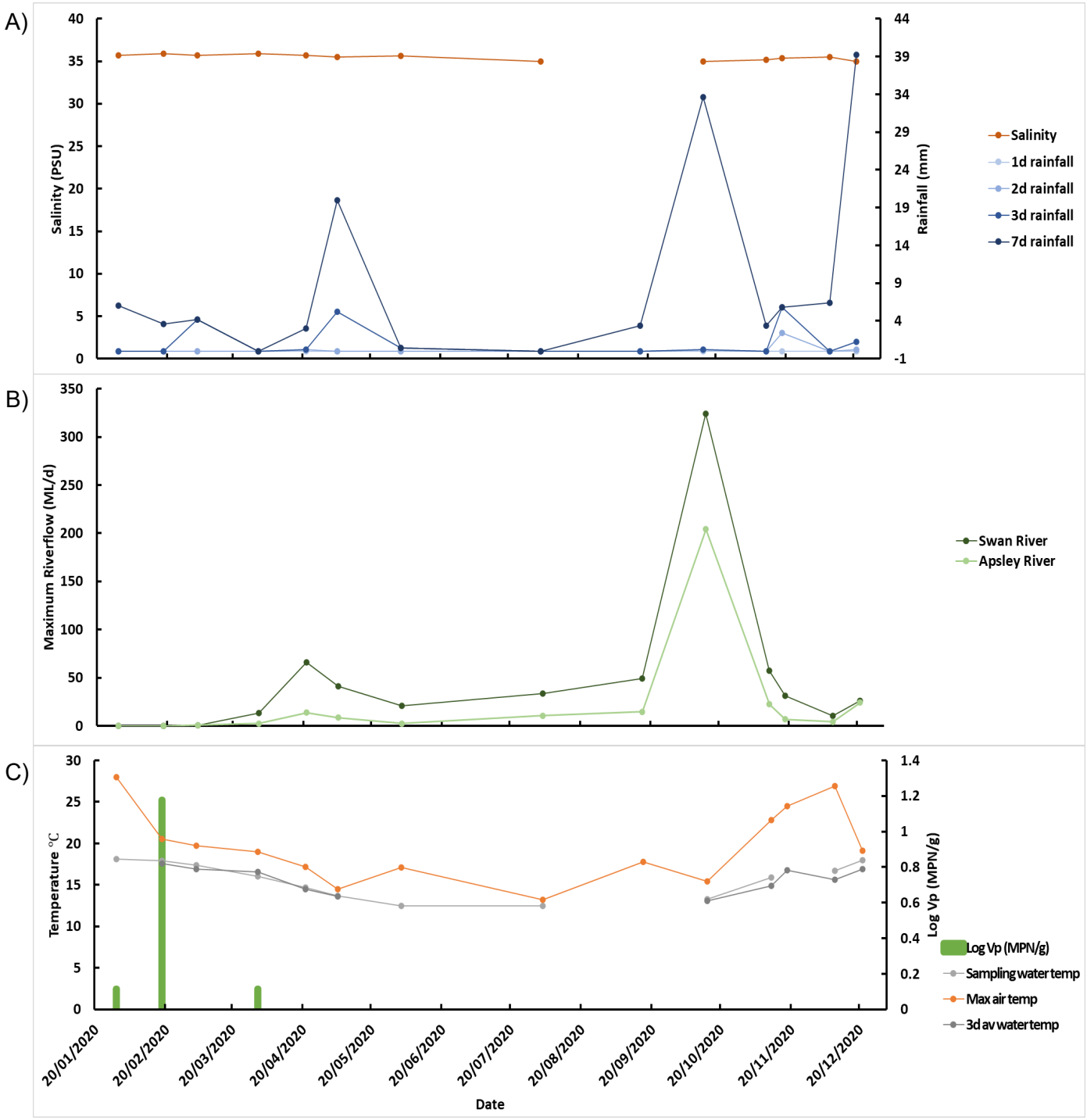

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Great Oyster Bay. 2A shows water salinity at sampling and rainfall (1, 2, 3 and 7 days) prior to sampling. 2B shows maximum river flow of two rivers 24 hours prior to sampling. 2C shows levels of *V. parahaemolyticus* detected and are overlayed with sampling water temperature, three-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence for Great Oyster Bay broken down to the 3 seasons sampled during the survey. *V. parahaemolyticus* was only detected in the first summer/autumn sampling period at a prevalence of 60%. No *V. parahaemolyticus* was detected in the second summer/autumn sampling period, although it should be noted that only 4 samples were collected and may not be representative of the season. No *V. parahaemolyticus* strains containing either the *tdh* or *trh* genes were detected. No *V. parahaemolyticus* was detected in the single winter/spring sampling period in 2020. *V. vulnificus* was detected in the first summer/autumn sampling period with a prevalence of 20%.

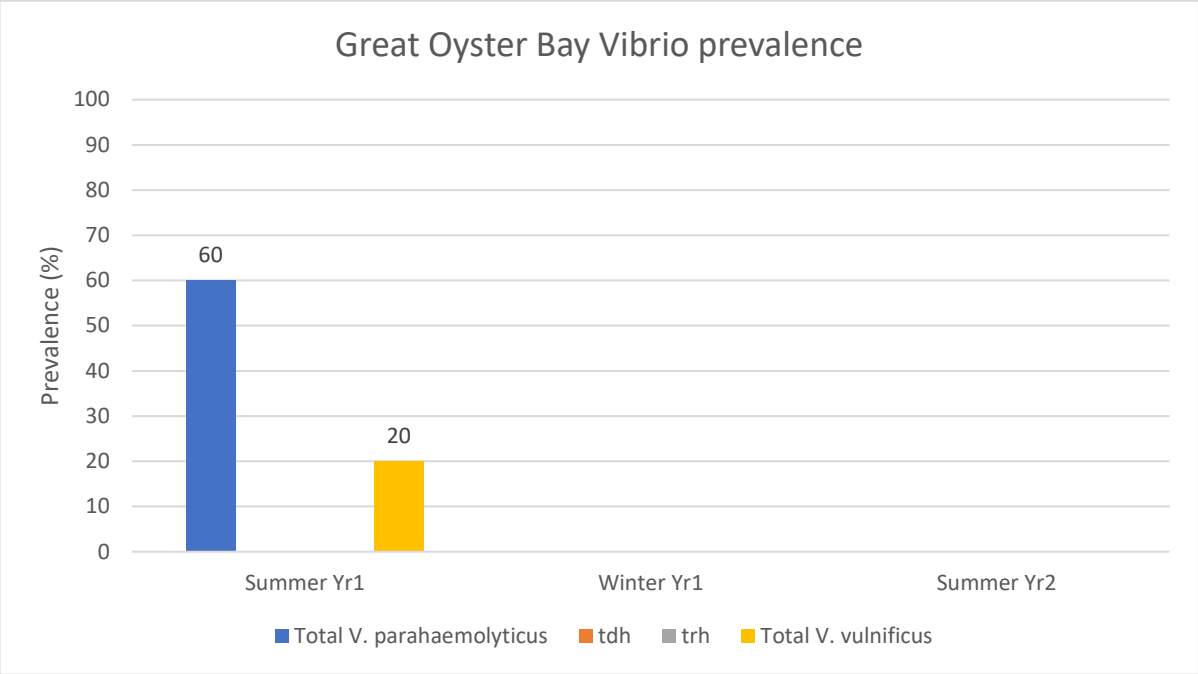

Figure 3: Seasonal *Vibrio* prevalence during the survey period.

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data during the survey aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of environmental conditions were recorded in the growing area over the period of the survey. *V. parahaemolyticus* and *V. vulnificus* numbers where low in non-abused oysters; ≤0.3 MPN/g.

Table 1: Summary table of environmental parameters over the summer/autumn and winter/spring survey periods

	Summer (n=5)					**Winter (n=5)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	16.0	18.1	17.2	1.0	4	12.5	13.7	13.0	0.6	4
Sampling air temp	NA	NA	NA	NA	0	NA	NA	NA	NA	0
3-day min H_2O temp	14.5	16.5	15.6	0.8	4	11.5	13.0	12.3	1.1	2
3-day max H_2O temp	16.5	17.0	16.8	0.3	4	13.5	14.0	13.8	0.4	2
3-day Av H_2O temp	15.6	16.9	16.5	0.6	4	13.1	13.6	13.4	0.4	2
1-day max air temp	19.0	28.0	23.5	4.3	5	13.2	17.8	15.6	1.9	5
Salinity (PSU)										
Sampling H_2O	35.0	35.9	35.5	0.3	5	35.0	35.6	35.3	0.3	4
Rainfall (mm)										
1-day	0	0	0	0	5	0	0	0	0	5
2-days	0	2.4	0.5	1.1	5	0	0.2	<0.1	0.1	5
3-days	0	5.8	1.4	2.5	5	0	5.2	1.2	2.3	5
7-days	0	39.2	11.5	15.7	5	0	33.6	11.5	14.8	5
River flow (ML/d)										
Swan River	<0.1	31.1	16.3	12.5	5	21.0	324.2	93.9	129.2	5
Apsley River	0	24.2	7.5	9.6	5	2.6	204.1	48.1	87.3	5

NA = not applicable

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods

***Vibrio* enumeration (MPN/g)**						
Vibrio	**Summer (n=5)**			**Winter (n=5)**		
	Min	**Max**	**Positive (n=)**	**Min**	**Max**	**Positive (n=)**
Total *V. parahaemolyticus*	0	0.3	2	0	0	0
tdh	0	0	0	0	0	0
trh	0	0	0	0	0	0
Total *V. vulnificus*	0	0.3	1	0	0	0

Conclusions

- Limited sampling occurred in Great oyster Bay (n=14), covering parts of the first and second summer/autumn and first winter/spring sampling periods in 2020. Furthermore, four of the submitted samples were potentially temperature abused during transit and hence were excluded from further analysis. Water temperature data was also limited as assigned loggers were not placed into the growing water. The only full environmental predictor data which we could obtain was for maximum daily air temperature, rainfall and river flow, all from the BOM and NRE portal.
- *V. parahaemolyticus* was detected in Great Bay in n=2/10 samples from the first summer/autumn sampling period. Levels of bacteria detected were very low: 0.3 MPN/g of oysters. The potential pathogenicity associated genes *tdh* and *trh* were not detected in these two positive samples.
- *V. vulnificus* was detected in one sample from the first summer/autumn sampling period: 0.3 MPN/g of oyster.
- Due to the limited sampling and accompanying environmental data particularly regarding water temperature no definite *V. parahaemolyticus* risk factors could be identified for Great Oyster Bay. In most other areas harvest water temperature was a significant risk factor, either in isolation or in combination with other environmental factors.

Appendix 6: *Vibrio* Survey Little Swanport, 2020-2022

Harvest area

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Little Swanport (Figure 1).

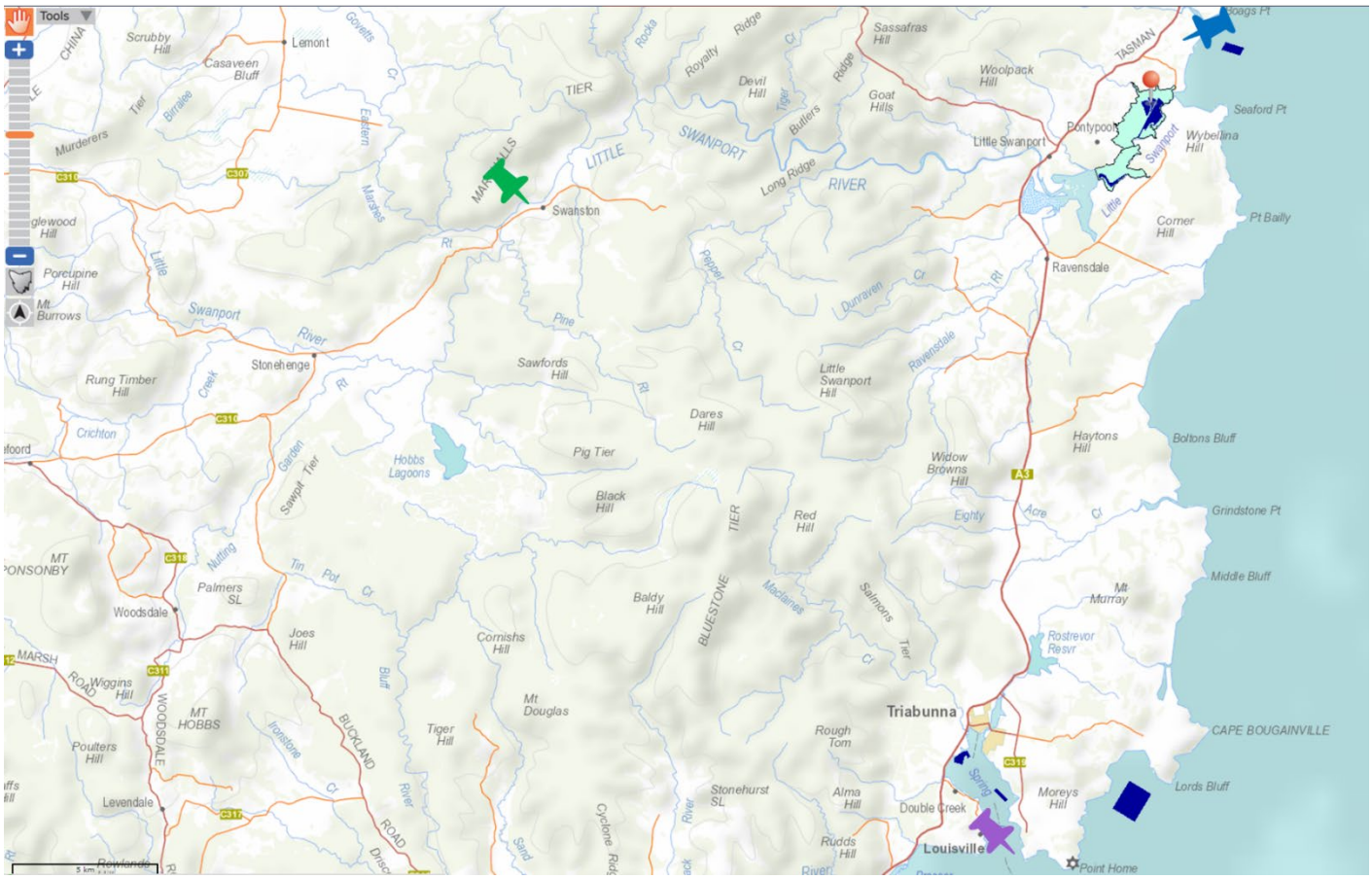

Figure 1: Little Swanport harvest area. Orange pin indicates lease from which sampling occurred. Green pin indicates Little Swanport River flow station. Purple pin indicates BOM temperature station at Spring Bay NTC AWS. Blue pin indicates BOM rainfall station at Little Swanport - Lisdillon Farm. Scale bar = 5 km.

Methodology

Summary of survey design

Oysters were sampled over three summer/autumn (November to April) and two winter/spring (May to October) periods from January 2020 to end of April 2022. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data collected: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data collected: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity, water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation. Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station number 92133; Spring Bay NTC AWS). Maximum river flow on the day of sampling was also accessed from the Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable,

for the river identified in the harvest areas' sanitary survey. For Little Swanport harvest area this was the Little Swanport River. Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 92154; Little Swanport - Lisdillon Farm).

Statistical analysis

Statistical analysis of biological and environmental data was done in Primer 7 + PERMANOVA (Primer-e). Correlation among variables was investigated using draftsman's plots. *V. parahaemolyticus* (MPN/g) data were $\log_{10}(X+1)$ transformed and used to create a Euclidean distance resemblance matrix. The environmental data was also transformed if not normally distributed: rainfall data for Little Swanport was square root transformed while river flow data was fourth root transformed. The DISTLM (distance-based linear model) routine was used to investigate the relationship between the *V. parahaemolyticus* Euclidean distance resemblance matrix and the environmental (temperature, salinity and rainfall) predictor variables. The step-wise "selection procedure" was used with the Akaike Information Criterion corrected for small sample sizes (AICc) "selection criterion" and 9999 permutations. AICc is a mathematical method for evaluating how well a model fits the data it was generated from. The output of this analysis gives a statistical marginal test for each individual predictor variable considered alone, and the best overall solution. The best linear model was visually represented using dbRDA (distance-based redundancy analysis). The formula for the resulting predictive model was generated using the regression function in excel.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Salinity and rainfall data during the survey period are shown in Figure 2A. The highest rainfall events were recorded in summer months. Rainfall did not always result in a strong flow in the Little Swanport River: flow was greatest in third summer following a long period of heavy rainfall. (Figure 2B). Figure 2C shows *V. parahaemolyticus* levels, sampling water temperature and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Temperature and *V. parahaemolyticus* numbers increased during summer/autumns months. Salinity in Little Swanport was variable (2-37 PSU) and some dramatic drops in salinity corresponded with increased rainfall and river flow.

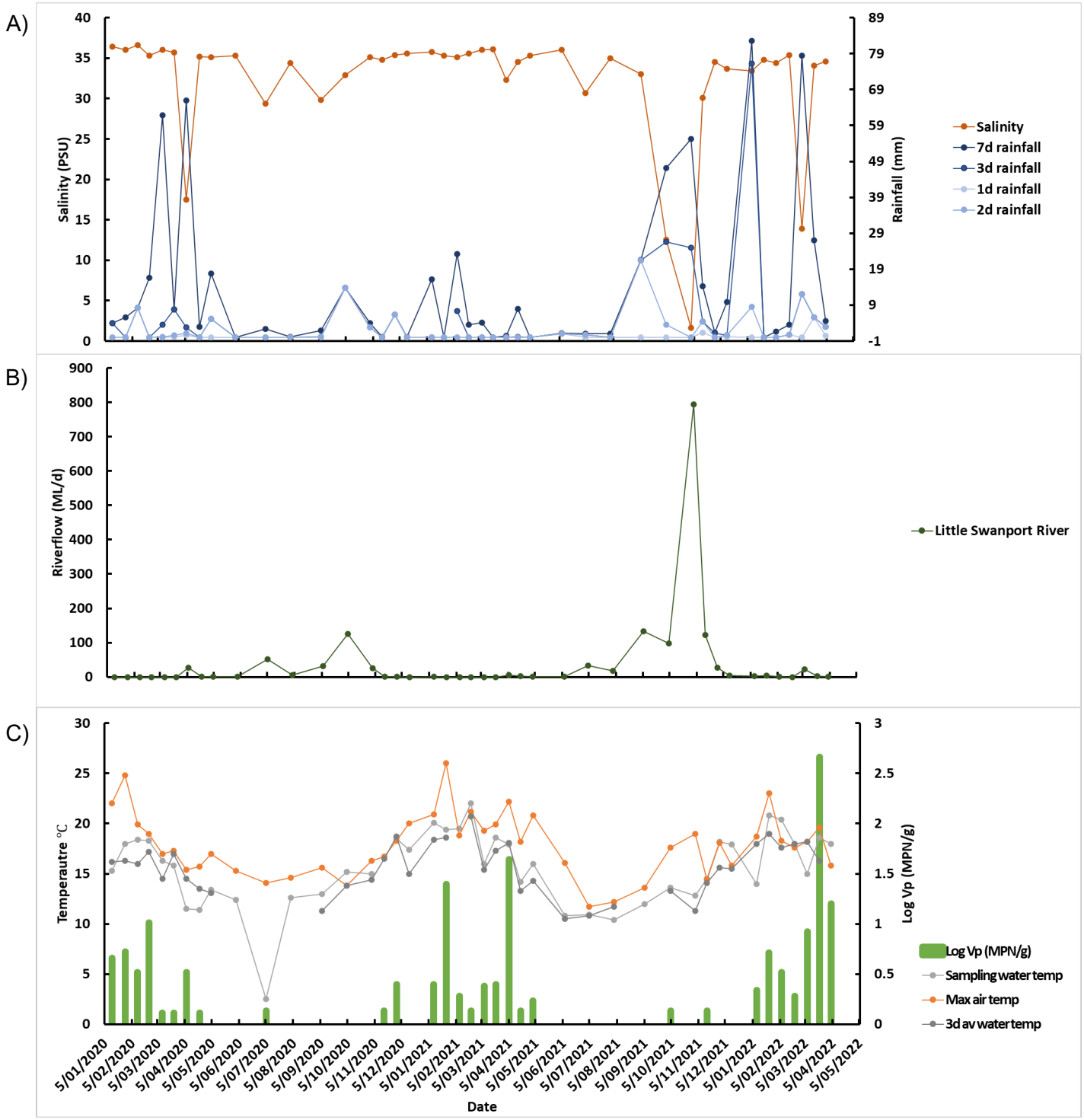

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Little Swanport. 2A shows water salinity at sampling and rainfall (1, 2, 3 and 7 days) prior to sampling. 2B shows maximum river flow 24 hours prior to sampling. 2C shows levels of *V. parahaemolyticus* detected and are overlayed with sampling water temperature, three-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence for Little Swanport broken down to the 5 seasons sampled during the survey. *V. parahaemolyticus* was detected all year round, but a higher prevalence was observed in the summer/autumn sampling periods (73-100%) as compared with the winter/spring sampling periods (17-33%). *V. parahaemolyticus* strains containing the pathogenicity associated genes *tdh* or *trh* were detected in the first and third summers with a prevalence of 9 and 13% respectively. *V. vulnificus* was only detected in the summer/autumn of year 3 with a prevalence of 27%.

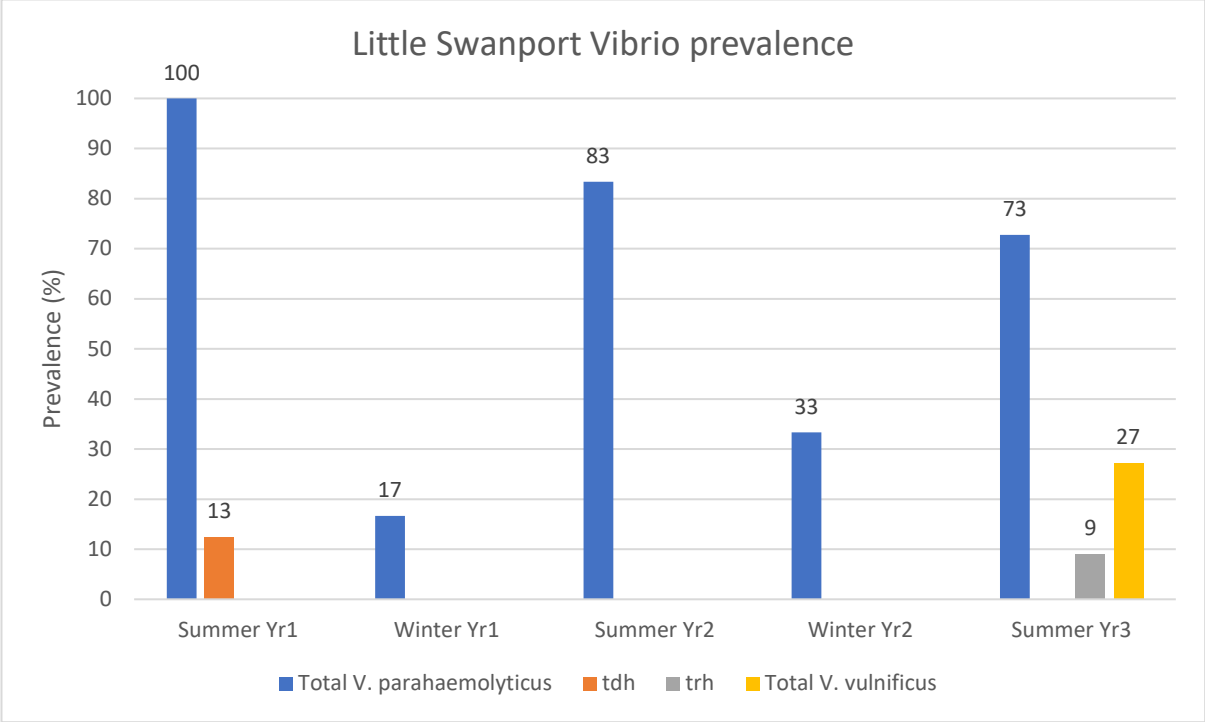

Figure 3: Seasonal *Vibrio* prevalence during the survey period.

V. parahaemolyticus was not detected in samples taken when sampling water temperature was <11°C, minimum and average water temperature 3-days before sampling were <7°Cand <13°C respectively, or when the max daily air temperature did not exceed 14°C. *V. parahaemolyticus* was detected in oysters collected both above (n=12) and below (n=15) the water. Of the 41 samples analysed 22 were collected below the water, 17 above the water and 2 not recorded. No relationship could be deduced between tidal stage an *V. parahaemolyticus* (Figure 4).

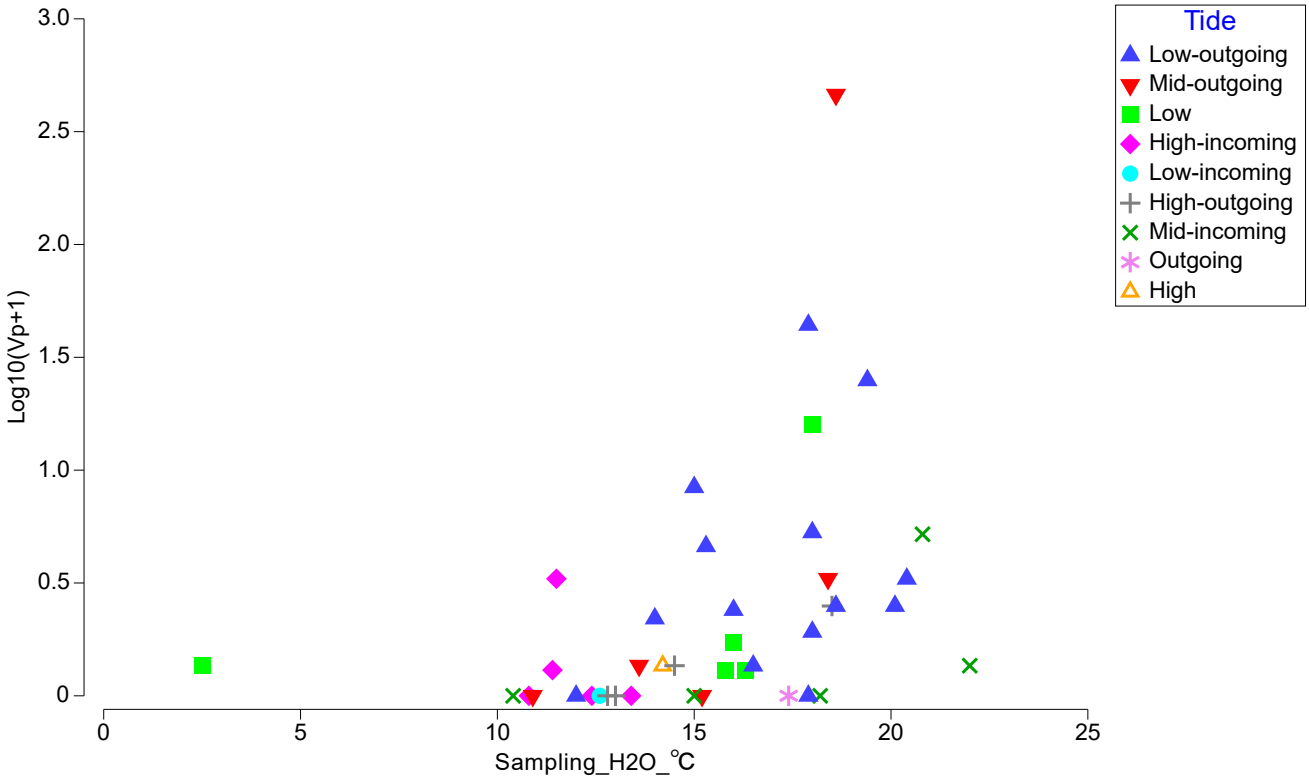

Figure 4: Scatter plot of *V. parahaemolyticus* versus sampling temperature identified by tidal stage of collection.

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data during the survey aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of environmental conditions were recorded in the growing area over the period of the survey. *V. parahaemolyticus* numbers where low to moderate, with a maximum detection of 460 MPN/g. Levels of *V. parahaemolyticus* carrying the pathogenicity determinants *tdh* or *trh* were very low (≤3.6 MPN/g). Levels of *V. vulnificus* detected were <1 MPN/g.

Table 1: Summary table of environmental parameters over the summer/autumn and winter/spring survey periods

	Summer (n=29)					**Winter (n=12)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	11.4	22.0	16.9	2.6	29	2.5	16.0	11.9	3.4	12
Sampling air temp	9.0	24.5	17.3	4.0	15	8.0	20.0	13.6	4.3	6
3-day min H_2O temp	7.0	17.0	11.7	2.8	28	5.0	12.0	8.7	2.8	8
3-day max H_2O temp	15.5	26.5	20.2	2.9	28	12.5	17.0	14.9	1.7	8
3-day Av H_2O temp	11.3	20.7	16.4	2.1	28	10.5	14.3	12.4	1.5	8
1-day max air temp	14.4	26.0	19.0	2.8	29	11.7	20.8	15.2	2.5	12
Salinity (PSU)										
Sampling H_2O	1.6	36.6	32.4	7.8	29	12.5	36.0	31.6	6.4	12
Rainfall (mm)										
1-day	0	8.2	0.9	2.1	29	0	13.8	1.2	4.0	12
2-days	0	12.0	1.9	3.2	29	0	21.4	3.8	6.8	12
3-days	0	76.2	5.7	14.5	29	0	26.6	5.8	9.5	12
7-days	0	82.6	16.5	25.3	29	0	47.2	9.0	14.3	12
River flow (ML/d)										
Little Swanport River	0	794	36.2	147.6	29	0.6	133.2	41.6	49.8	12

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods

***Vibrio* enumeration (MPN/g)**						
Vibrio	**Summer (n=29)**			**Winter (n=12)**		
	Min	**Max**	**Positive (n=)**	**Min**	**Max**	**Positive (n=)**
Total *V. parahaemolyticus*	0	460	24	0	0.72	3
tdh	0	3.6	1	0	0	0
trh	0	0.3	1	0	0	0
Total *V. vulnificus*	0	0.72	3	0	0	0

Statistical analysis

Temperature

The temperature predictor variables were highly correlated with one another (Figure 5). The water temperature variables were more highly correlated with one another than with the air temperature predictor variable. *V. parahaemolyticus* numbers were positively correlated with temperature; most strongly correlated (R=0.51) with the maximum daily air temperature on the day of sampling, followed by the maximum and average water temperatures 3-days before sampling (Figure 5).

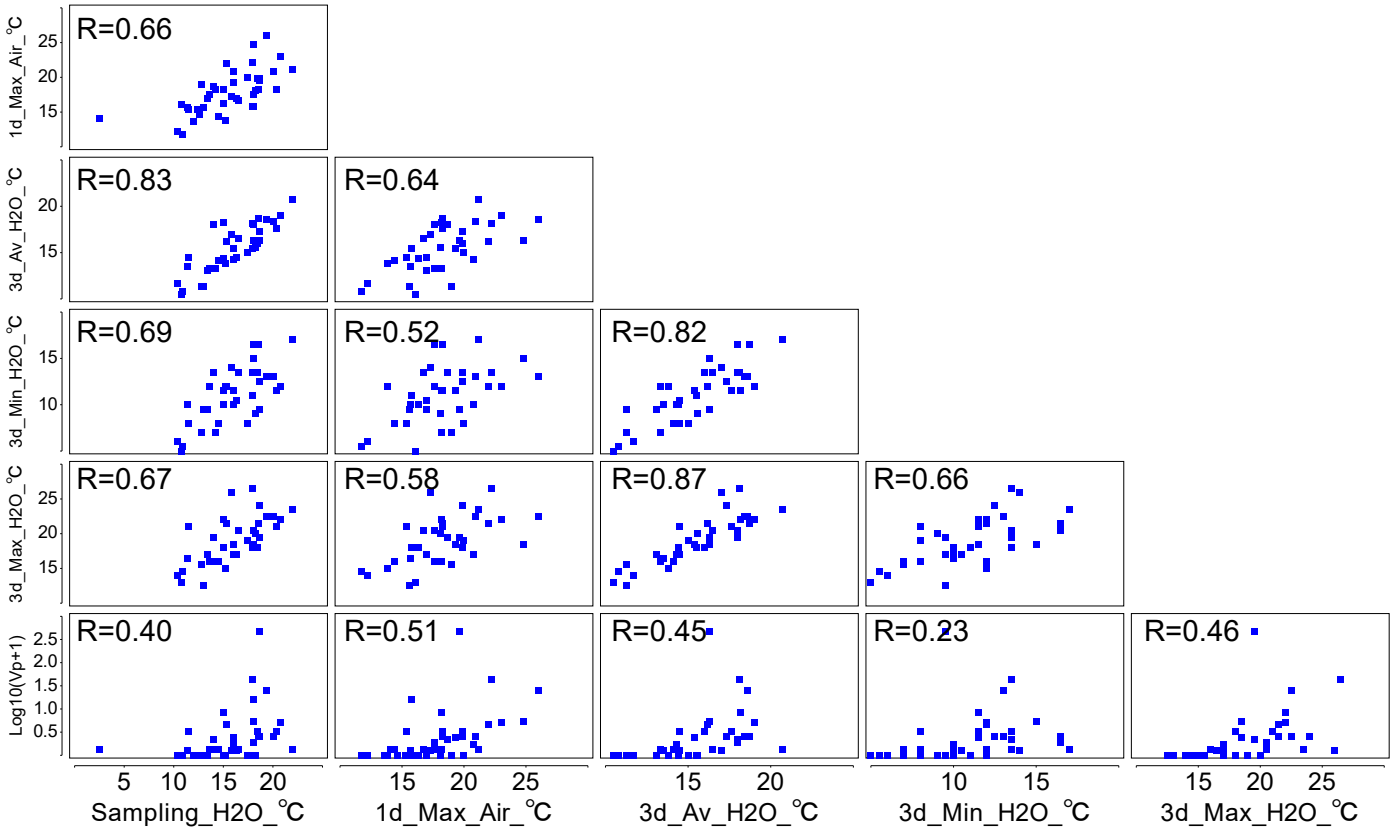

Figure 5: Draftsman's plots of temperature variables and *V. parahaemolyticus*.

Rainfall

Rainfall 1 to 7-days preceding sampling were positively correlated with one another (Figure 6). The highest inter-rainfall correlations were observed between 3- and 7-days, followed by 2- and 3-days. *V. parahaemolyticus* numbers were not correlated with rainfall, with the highest correlation observed with 1 day rainfall (R=0.18).

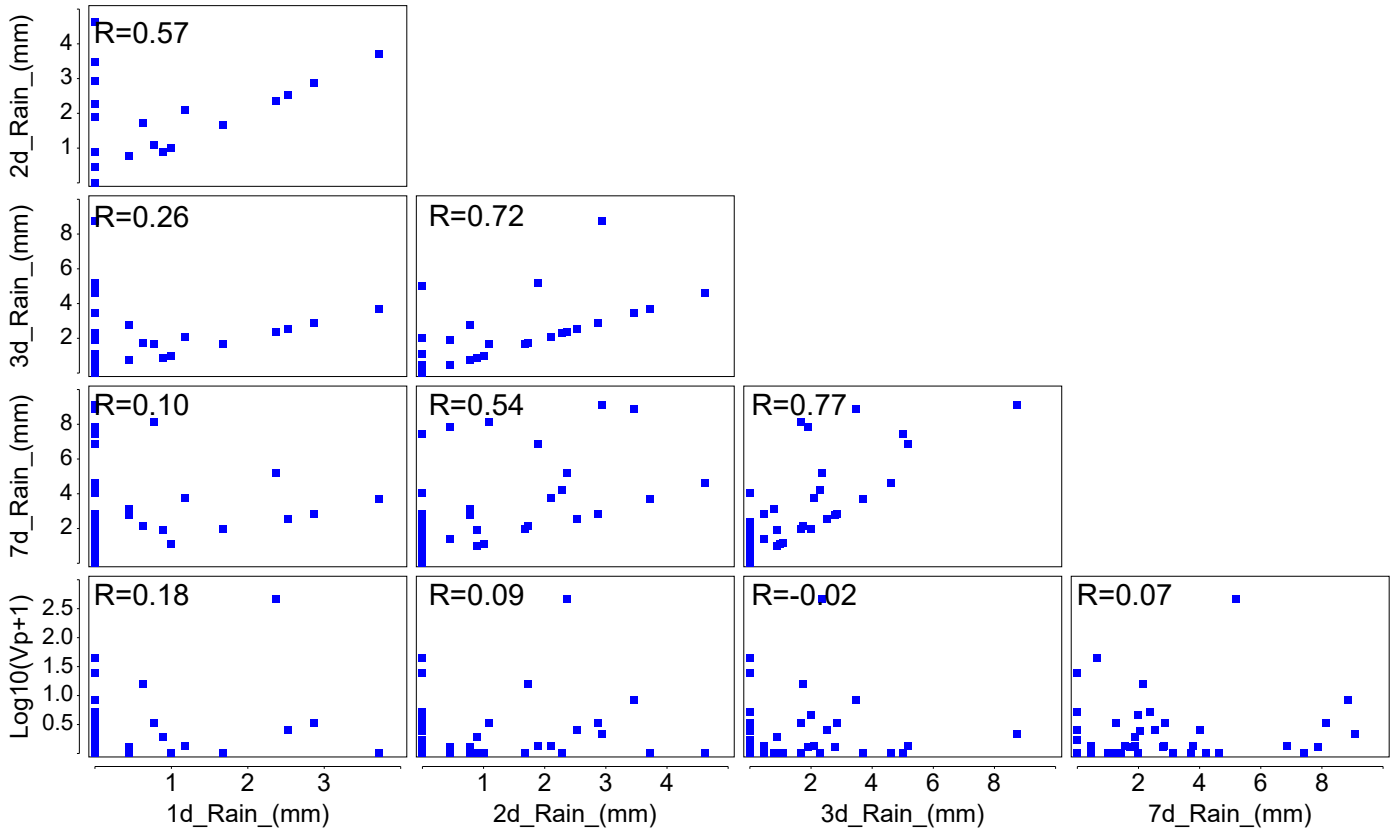

Figure 6: Draftsman's plots of rainfall variables and *V. parahaemolyticus*. Rainfall data was square root transformed.

Salinity and river flow

Harvest water salinity and river flow were negatively correlated for Little Swanport (Figure 7). *V. parahaemolyticus* levels were poorly correlated with harvest water salinity and somewhat negatively correlated with river flow (R=-0.25).

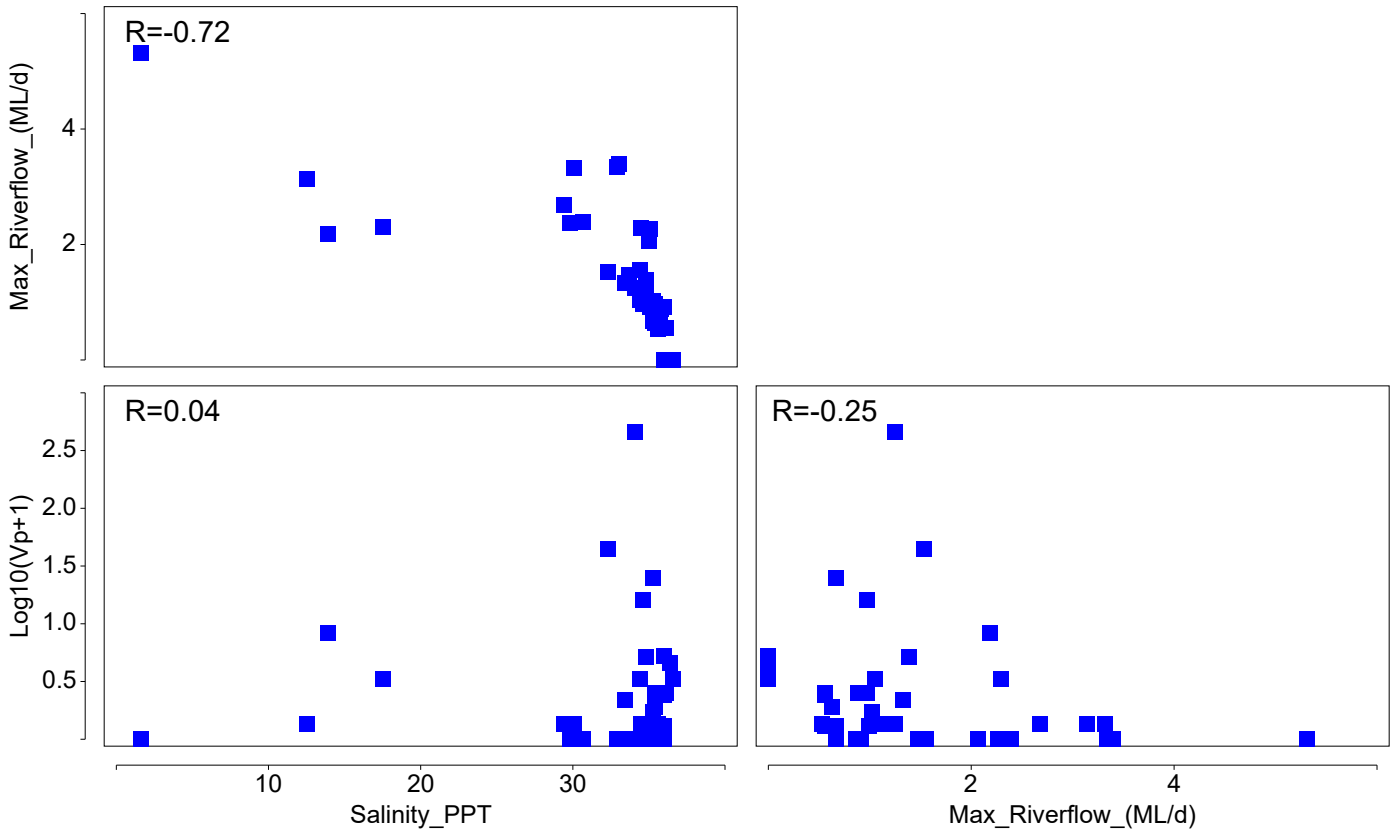

Figure 7: Draftsman's plots of salinity, river flow and *V. parahaemolyticus* variables. River flow data was fourth root transformed.

Linear predictive model generation

The marginal statistical tests in DISTLM showed significant relationships of various temperature predictor variables with *V. parahaemolyticus* numbers. Sampling water temperature (Pseudo-F=6.0, P=0.017), average (Pseudo-F=8.6, P=0.004) and maximum (Pseudo-F=8.9, P=0.003) water temperature 3-days prior to sampling, as well as, the maximum daily air temperature (Pseudo-F=13.1, P=0.002) were all found to be significant drivers individually, explaining 15%, 20%, 21% and 28% of the observed variability, respectively. All these temperature variables were noted to be highly correlated (Figure 5) and after fitting maximum daily air temperature, 2-day rainfall explained an additional 8% of the variability. These two predictor variables described 100% of the explained variability in the fitted model, 36% of the total variability in the data cloud (AICc= -52.922 R^2=0.36) (Figure 8).

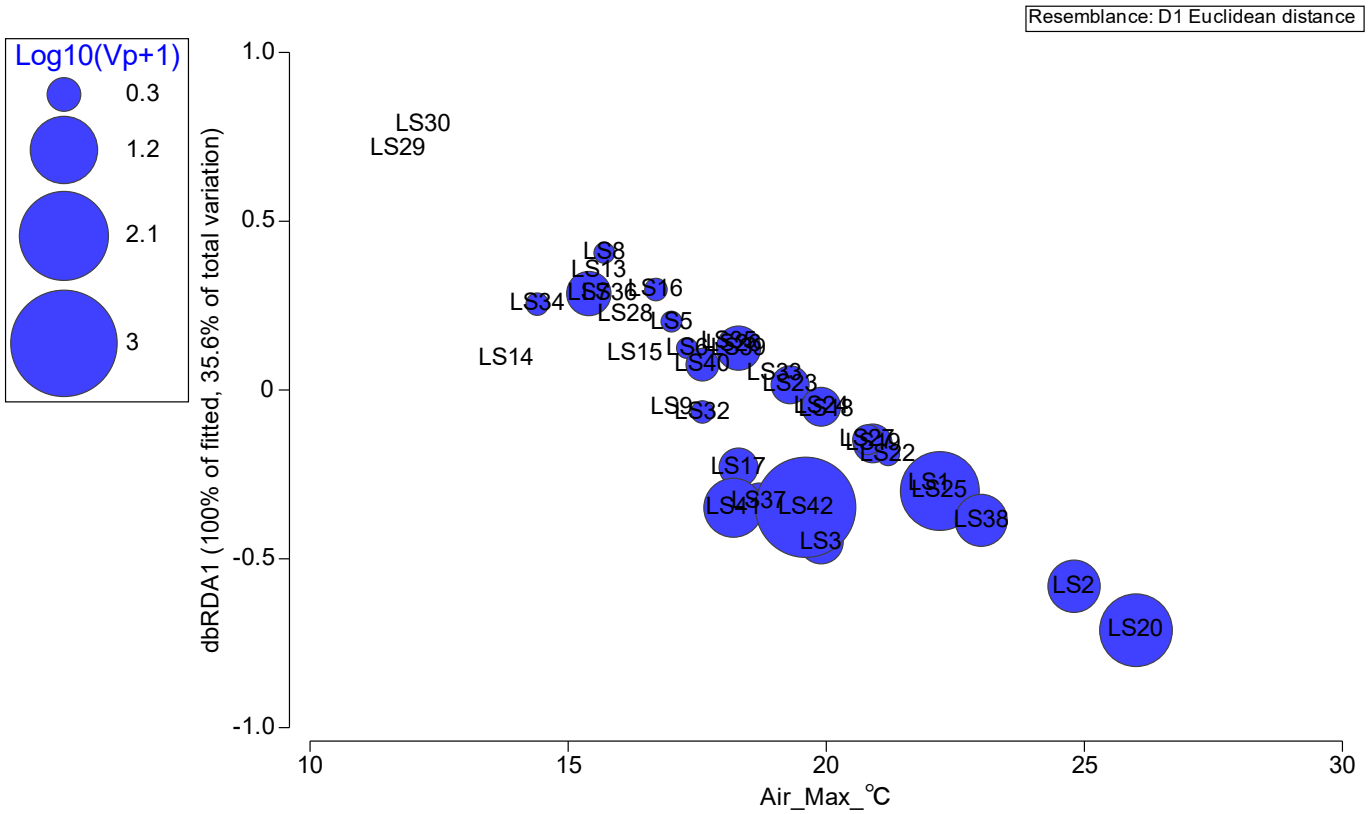

Figure 8: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with the predictor variables of maximum daily air temperature and rainfall 2-days prior to sampling. Bubbles on plot indicate level of *V. parahaemolyticus* (Vp) detected in shellfish sampled from Little Swanport.

The predictive model developed for *V. parahaemolyticus* in Little Swanport for oysters was based on the maximum daily air temperature and 2-day rainfall prior to sampling:

$$y = -1.7285 + 0.1085x_0 + 0.1396\sqrt{x_1}$$

Where:

y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = maximum daily air temperature (°C)
x_1 = 2-day rainfall prior to sampling (mm)
R^2 = 0.36

Conclusions

- *V. parahaemolyticus* was detected in Little Swanport in all sampling seasons between 2020 and 2022, although the prevalence was higher (73-100%) in the summer/autumn seasons as compared with the winter/spring seasons (17-33%).
- Levels of *V. parahaemolyticus* detected during the survey period were low to moderate; ≤460 MPN/g of oyster.
- *V. parahaemolyticus* carrying the *tdh* and *trh* genes (often associated with clinical strains) were only detected in the summer/autumn seasons with a prevalence of 13% for *tdh* in the first summer/autumn and 9% for *trh* in the third summer/autumn seasons.
- *V. vulnificus* was only detected during the third summer/autumn sampling period with a prevalence of 13% and levels <1 MPN/g of oysters. Salinity of sampling water for these positive samples ranged from 30-35 PSU and sampling water temperature ranged from 15-18°C.
- Water and air temperature were individually good indicators of *V. parahaemolyticus* risk in oysters from Little Swanport. No *V. parahaemolyticus* was detected during the survey period when sampling water and the average water temperature 3 days preceding sampling were below 11°C and 13°C, respectively.
- The best predictive model developed for *V. parahaemolyticus* risk in Little Swanport incorporated both maximin daily air temperature and 2-day rainfall preceding harvest. No *Vibrio* was detected when the maximum daily air temperature was below 14°C.

$$y = -1.7285 + 0.1085x_0 + 0.1396\sqrt{x_1}$$

Where:
y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = maximum daily air temperature (°C)
x_1 = 2-day rainfall prior to sampling (mm)
R^2 = 0.36

Appendix 7: *Vibrio* Survey Boomer Bay, 2020-2022

Background

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Boomer Bay (Figure 1).

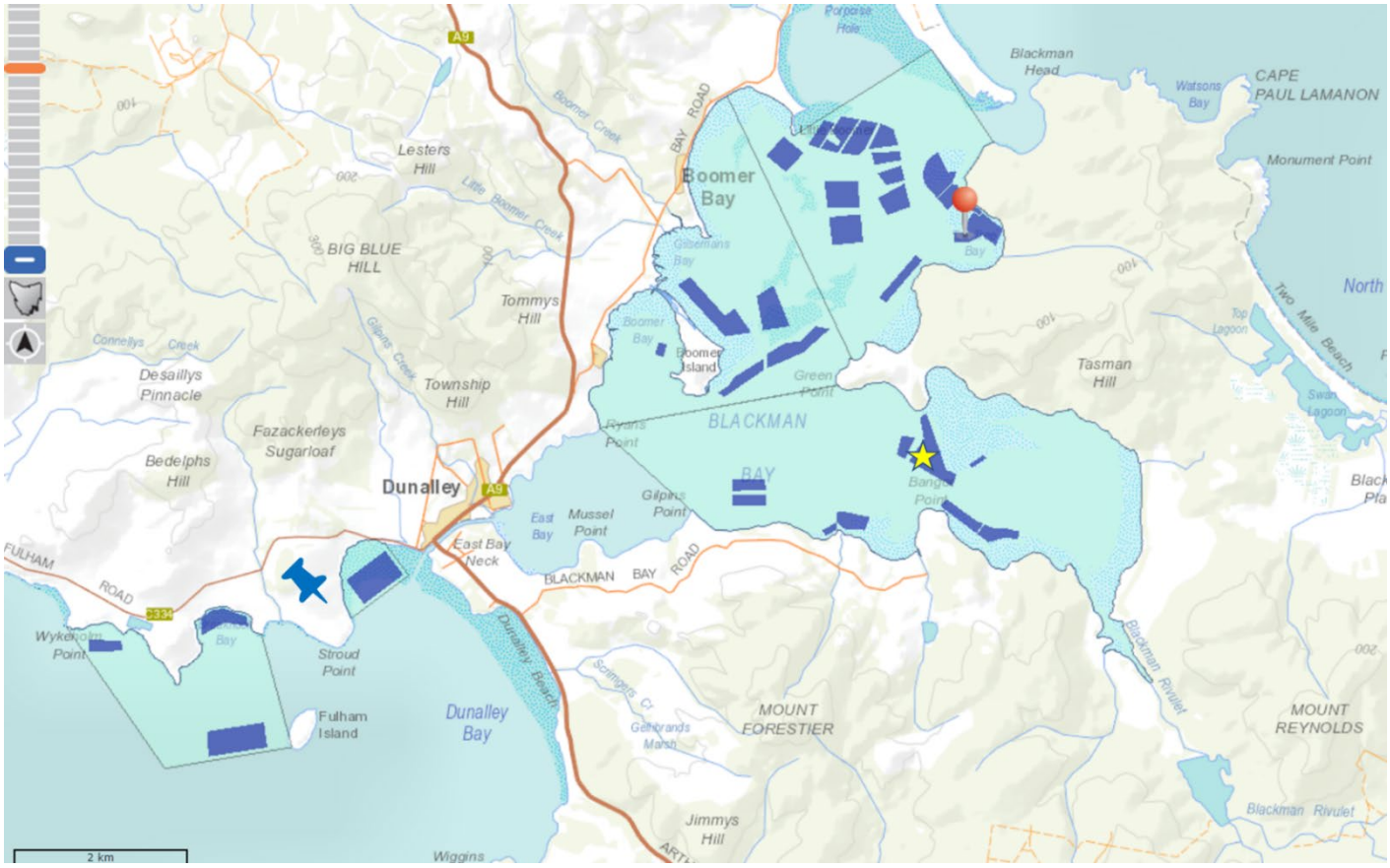

Figure 1: Boomer Bay harvest area. Orange pin indicates lease from which sampling occurred. Blue pin indicates BOM weather station at Dunalley. The decimal degree (DD) coordinates of the Boomer Bay lease is -42.85968, 147.87979. Yellow star indicates lease in Boomer Bay East (-42.88334, 147.87497). Scale bar = 2 km.

Methodology

Summary of survey design

Oysters were sampled over three summer/autumn (November to April) and two winter/spring (May to October) periods from January 2020 to end of April 2022. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data collected: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data collected: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity, water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation. Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station number 94254; Dunalley - Stroud Point). Maximum river flow on the day of sampling was also accessed from the Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable, for the river identified in the harvest area's sanitary survey. For Boomer Bay no river was identified. Rainfall and

maximum daily air temperature were taken from the same BOM station for both Boomer Bay and Boomer Bay East samples, although sampling generally occurred on different days. Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 94254; Dunalley - Stroud Point).

Statistical analysis

Statistical analysis of biological and environmental data was done in Primer 7 + PERMANOVA (Primer-e). Correlation among variables was investigated using draftsman's plots. *V. parahaemolyticus* (MPN/g) data were $\log_{10}(X+1)$ transformed and used to create a Euclidean distance resemblance matrix. The environmental data was also transformed if not normally distributed: rainfall data for Boomer Bay was square root transformed. The DISTLM (distance-based linear model) routine was used to investigate the relationship between the *V. parahaemolyticus* Euclidean distance resemblance matrix and the environmental (temperature, salinity, rainfall and river flow) predictor variables. The step-wise "selection procedure" was used with the Akaike Information Criterion corrected for small sample sizes (AICc) "selection criterion" and 9999 permutations. AICc is a mathematical method for evaluating how well a model fits the data it was generated from. The output of this analysis gives a statistical marginal test for each individual predictor variable considered alone, and the best overall solution. The best linear model was visually represented using dbRDA (distance-based redundancy analysis). The formula for the resulting predictive model was generated using the regression function in excel.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Salinity and rainfall data during the survey period are shown in Figure 2A. Larger volumes of rainfall occurred in the summer/autumn seasons. Rainfall didn't seem to impact salinity, which remained relatively constant throughout the year (33-36 PSU). Figure 2B shows *V. parahaemolyticus* levels, sampling water temperature, and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Higher *V. parahaemolyticus* levels seemed to track with warmer water and air temperatures in the summer/autumn seasons.

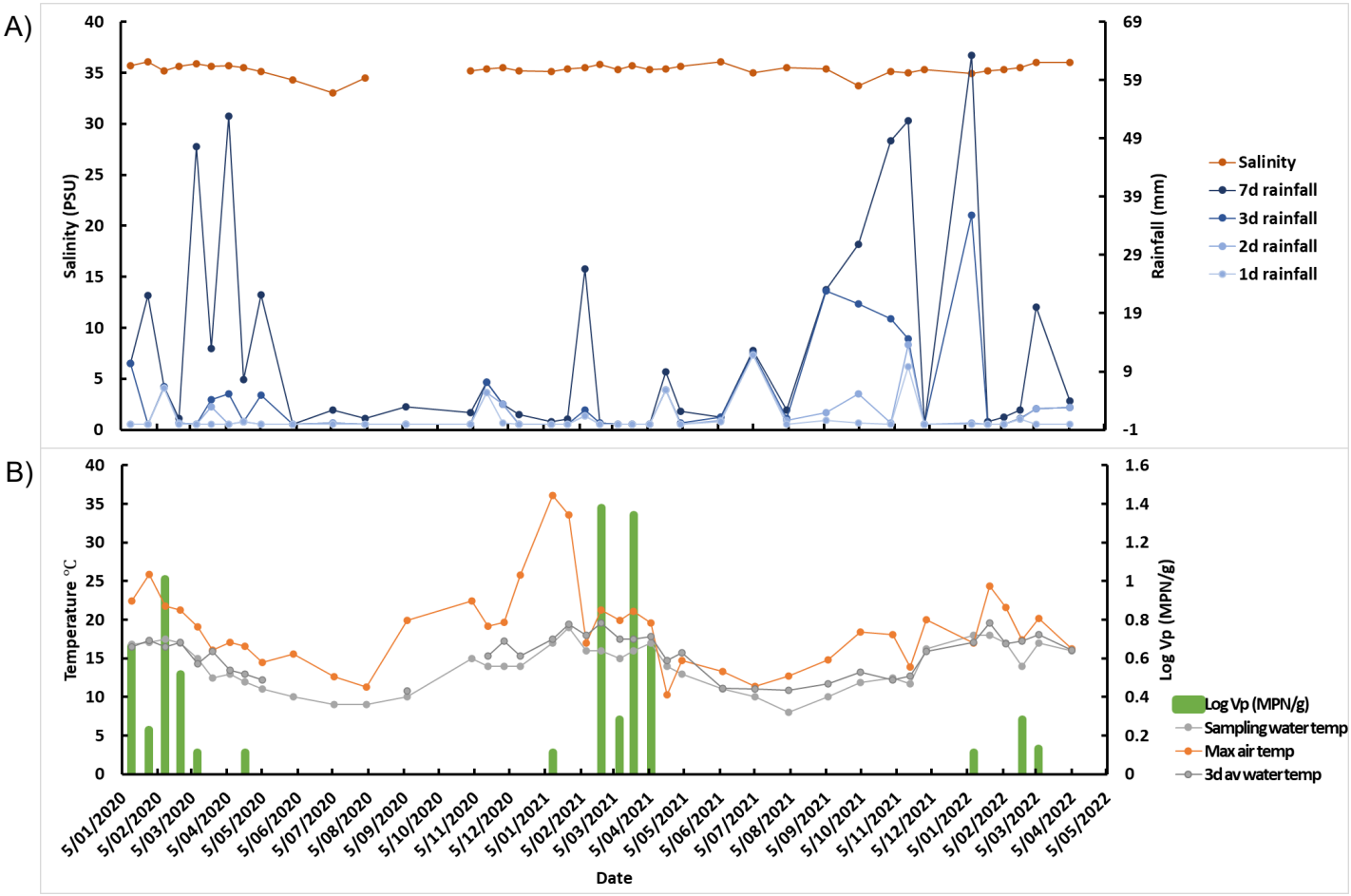

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Boomer Bay. 2A shows water salinity at sampling and rainfall at Dunalley (1, 2, 3 and 7 days) prior to sampling. 2B shows levels of *V. parahaemolyticus* detected overlayed with sampling water temperature, three-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence in Boomer Bay broken down to the 5 seasons sampled during the survey. High *V. parahaemolyticus* prevalence was observed in all summer/autumn sampling seasons (33-75%). No *V. parahaemolyticus* was detected in the winter/spring sampling seasons. *V. parahaemolyticus* containing the *tdh* and *trh* pathogenicity associated genes were only detected during the summer/autumn seasons; both genes detected in 13% of *V. parahaemolyticus* strains in the first summer/autumn sampling season and *tdh* only detected in 8% of *V. parahaemolyticus* strains during the second summer/autumn sampling season.

No *V. vulnificus* was detected in Boomer Bay during the 2020-2022 survey.

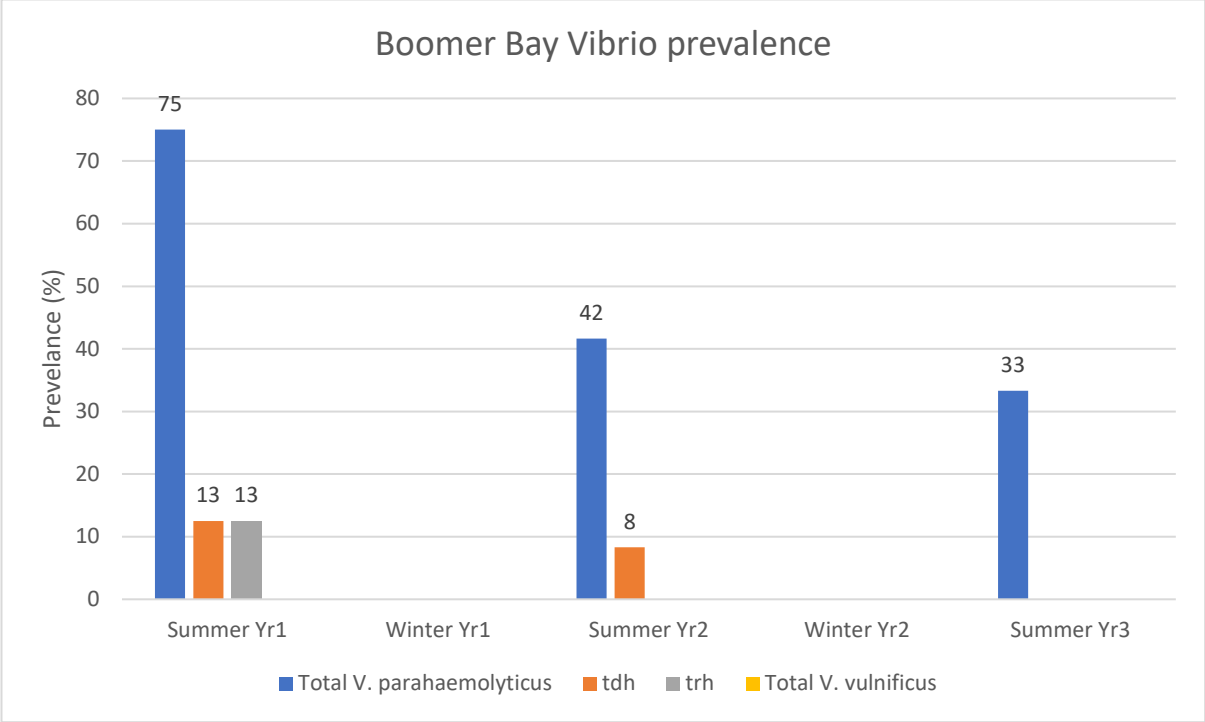

Figure 3: Seasonal *Vibrio* prevalence during the survey period.

V. parahaemolyticus was not detected when the sampling water temperature was <12°C, the 3-day average water temperature preceding sampling was <13°C or the minimum water temperature 3-days prior to sampling was <8°C.

Of the 31 samples collected during the survey period, n=28 were collected when oysters were below the water and 2 above the water (data for one sample was not recorded). No relationship could be deduced with tidal stage and *V. parahaemolyticus* levels (Figure 4). Most of the samples collected were at incoming (n=13) or high tidal stages (n=7).

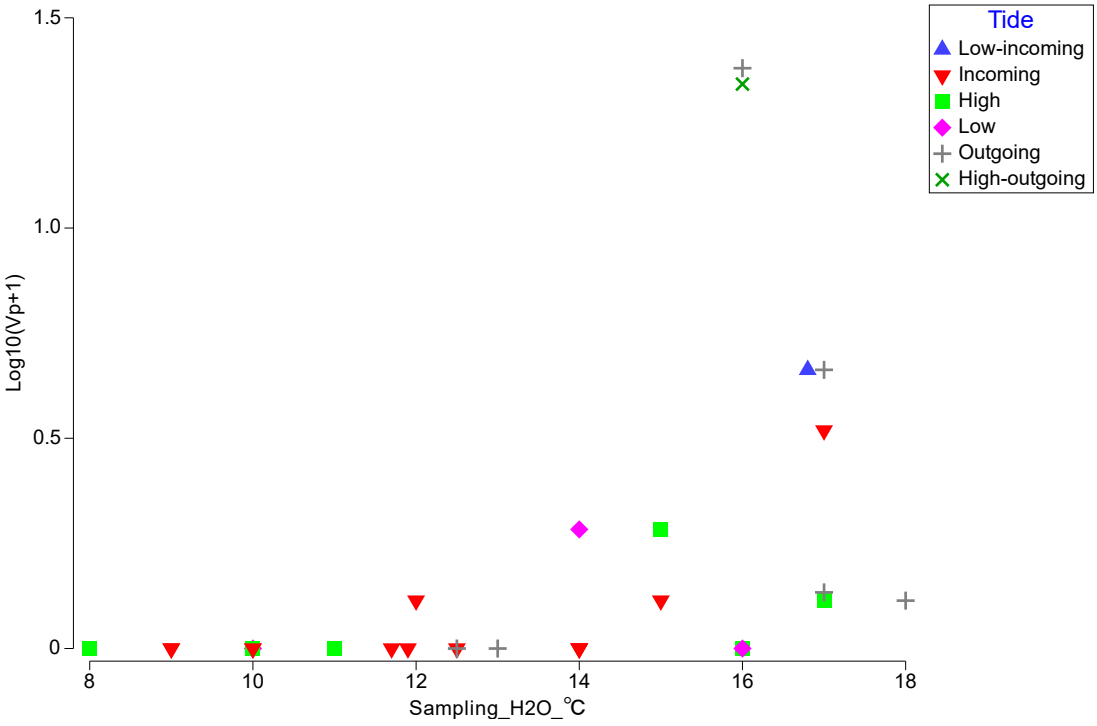

Figure 4: Scatter plot of *V. parahaemolyticus* versus sampling temperature identified by tidal stage of collection

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data during the survey, aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of environmental (temperature and rainfall) conditions were recorded during the survey although the sampling water salinity varied little (33-36 PSU). *V. parahaemolyticus* levels detected were generally low during the survey period (≤23 MPN/g in oyster).

Table 1: Summary table of environmental parameter over the summer/autumn and winter/spring survey periods.

	Summer (n=20)					**Winter (n=11)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	11.7	18.0	15.0	1.9	20	8.0	13.0	10.3	1.4	11
Sampling air temp	6.0	18.0	13.1	3.5	12	4.0	12.0	9.6	3.6	5
3-day min H_2O temp	6.5	16.5	12.8	2.8	20	5.5	12.0	8.4	2.0	8
3-day max H_2O temp	14.0	25.0	19.1	2.8	20	12.0	18.5	13.8	2.3	8
3-day Av H_2O temp	12.2	19.6	16.3	2.0	20	10.8	15.7	12.1	1.7	8
1-day max air temp	10.3	36.1	19.1	5.0	20	11.3	19.9	14.5	2.7	11
Salinity (PSU)										
Sampling H_2O	34.9	36	35.5	0.3	20	33	36.1	34.8	1.0	10
Rainfall (mm)										
1-day	0	9.8	1.2	2.6	20	0	11.8	1.2	3.5	11
2-days	0	13.6	2.0	3.3	20	0	11.8	1.9	3.6	11
3-days	0	35.8	5.5	8.8	20	0	22.8	5.7	8.7	11
7-days	0	63.2	15.8	20.4	20	0	30.8	9.2	11.1	11

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods.

***Vibrio* enumeration (MPN/g)**						
Vibrio	**Summer (n=20)**			**Winter (n=11)**		
	Min	**Max**	**Positive (n=)**	**Min**	**Max**	**Positive (n=)**
Total *V. parahaemolyticus*	0	23	12	0	0	0
tdh	0	3	2	0	0	0
trh	0	3	1	0	0	0
Total *V. vulnificus*	0	0	0	0	0	0

Statistical analysis

Temperature

The temperature predictor variables were all correlated (R>0.50) with one another (Figure 5), although the maximum daily air temperature was the least corelated with the other various water temperature variables (R<0.62). The 3-day average water temperature and maximum water temperature 3 days prior to sampling were the most correlated (R=0.96). *V. parahaemolyticus* numbers were most correlated with the average and maximum water temperature 3-days prior to shellfish sampling (R=0.55).

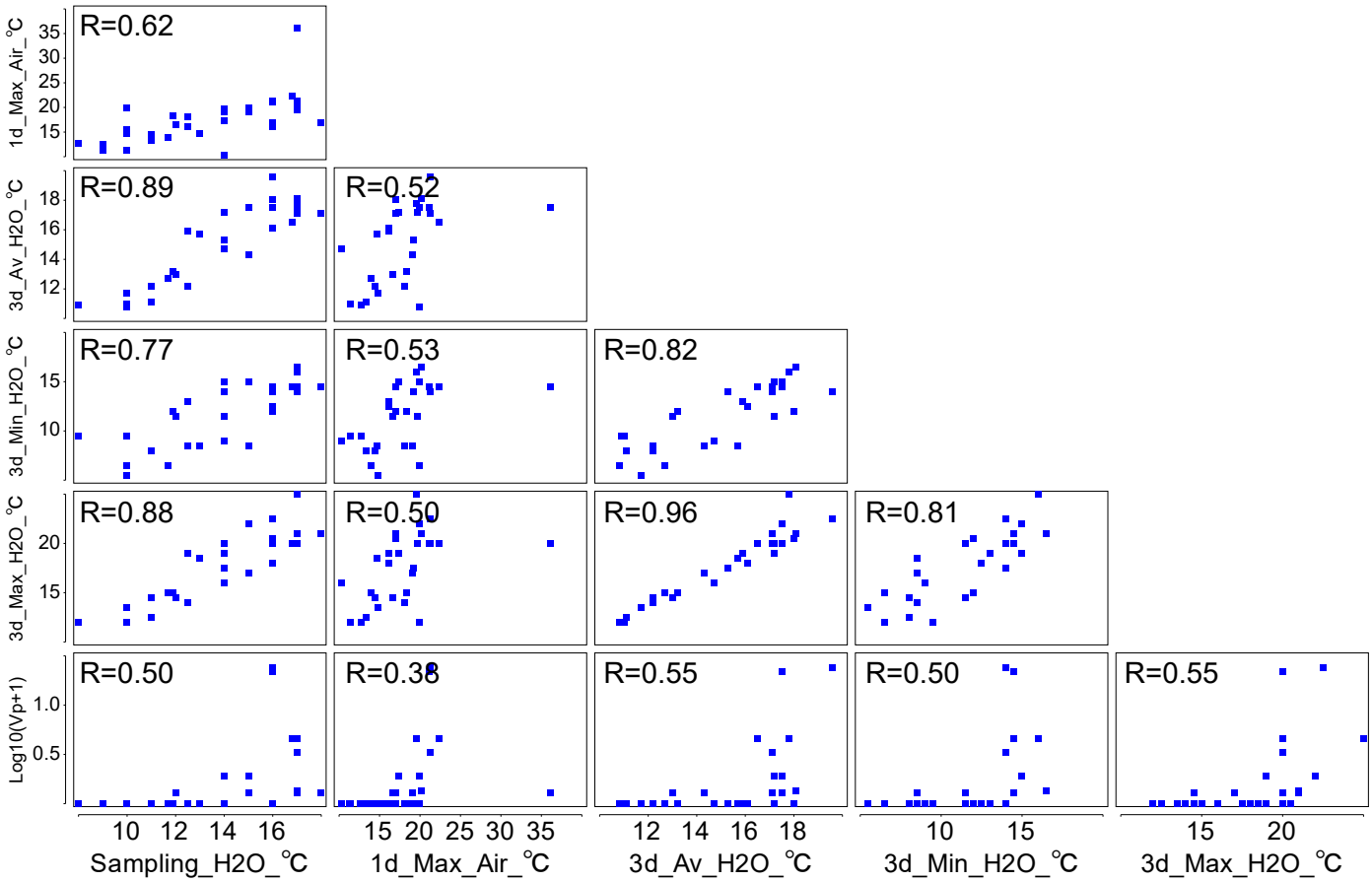

Figure 5: Draftsman's plots of temperature variables and *V. parahaemolyticus*.

Rainfall and salinity

Salinity was poorly correlated with rainfall (Figure 6). Salinity was somewhat positively correlated with *V. parahaemolyticus* numbers. Rainfall measures were somewhat correlated with one another, with the highest correlation observed between 2- and 3-day rainfall. *V. parahaemolyticus* numbers were somewhat negatively correlated with rainfall, with the highest correlation observed with 7-day rainfall.

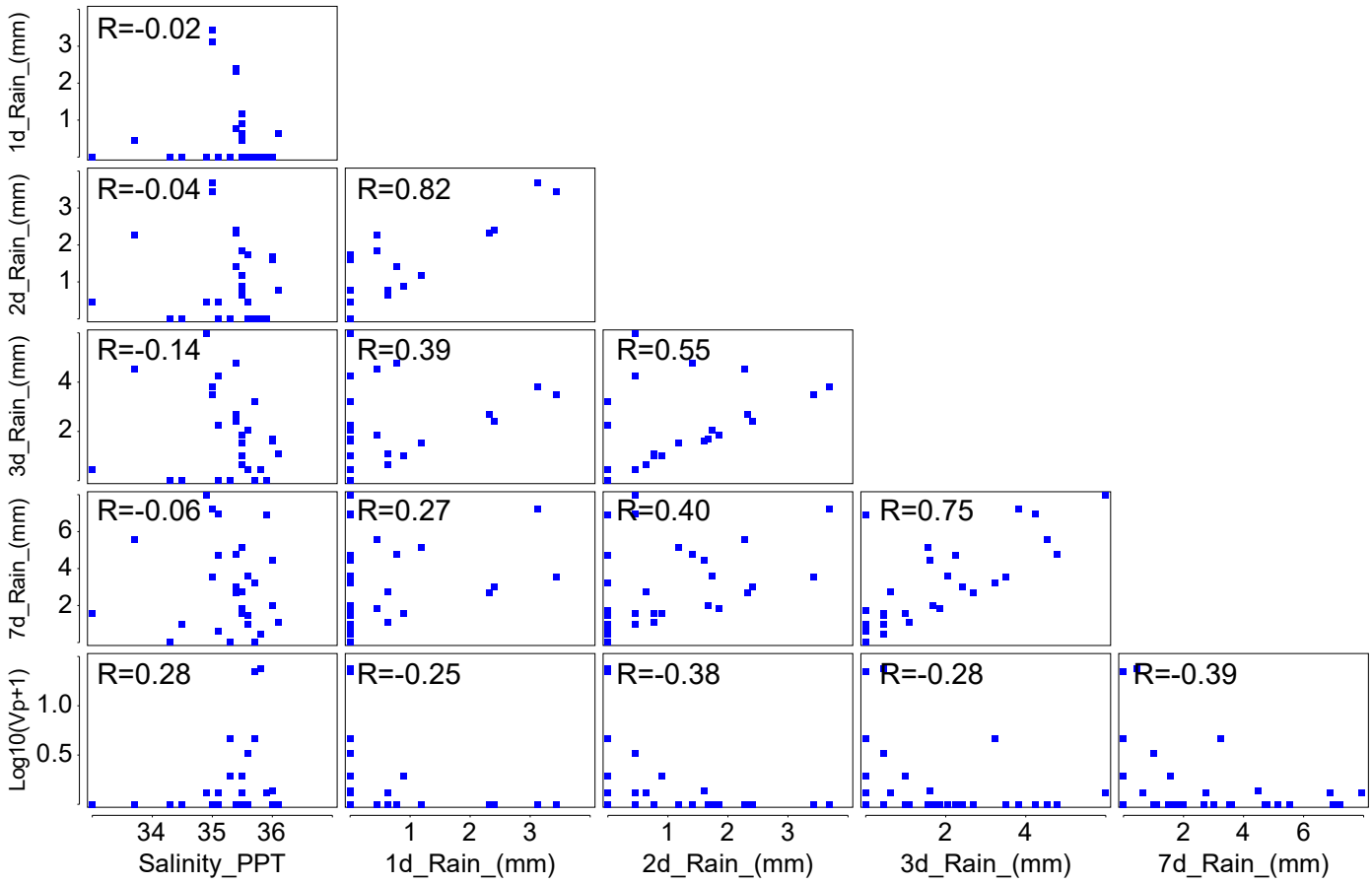

Figure 6: Draftsman's plots of rainfall and salinity variables and *V. parahaemolyticus*.

Linear model generation

Marginal statistical tests in DISTLM showed a significant relationship between *V. parahaemolyticus* and all of the water temperature predictor variables: sampling water temperature (Pseudo-F=7.2, P=0.010); 3-day average water temperature (Pseudo-F=10.7, P=0.002); minimum water temperature (Pseudo-F=8.0, P=0.005); and maximum water temperature (Pseudo-F=10.5, P=0.001) explaining 22%, 30%, 24% and 30% of the individual variation respectively. All these water temperature variables were highly correlated. The marginal statistical tests also showed a significant relationship between *V. parahaemolyticus* and rainfall: 2-day rainfall (Pseudo-F=7.0, P=0.014); 3-day rainfall (Pseudo-F=4.4, P=0.041); and 7-day rainfall (Pseudo-F=7.4, P=0.008) explaining 22%, 15% and 23% of the individual variation respectively. Three and 7-day rainfall were strongly correlated. However, after fitting the 3-day average water temperature prior to sampling, 7-day rainfall explained an additional 9% of the variability. These two predictor variables described 100% of the explained variability in the fitted model, 39% of the total variability in the data cloud (AICc=-58.702 R^2=0.39) (Figure 7).

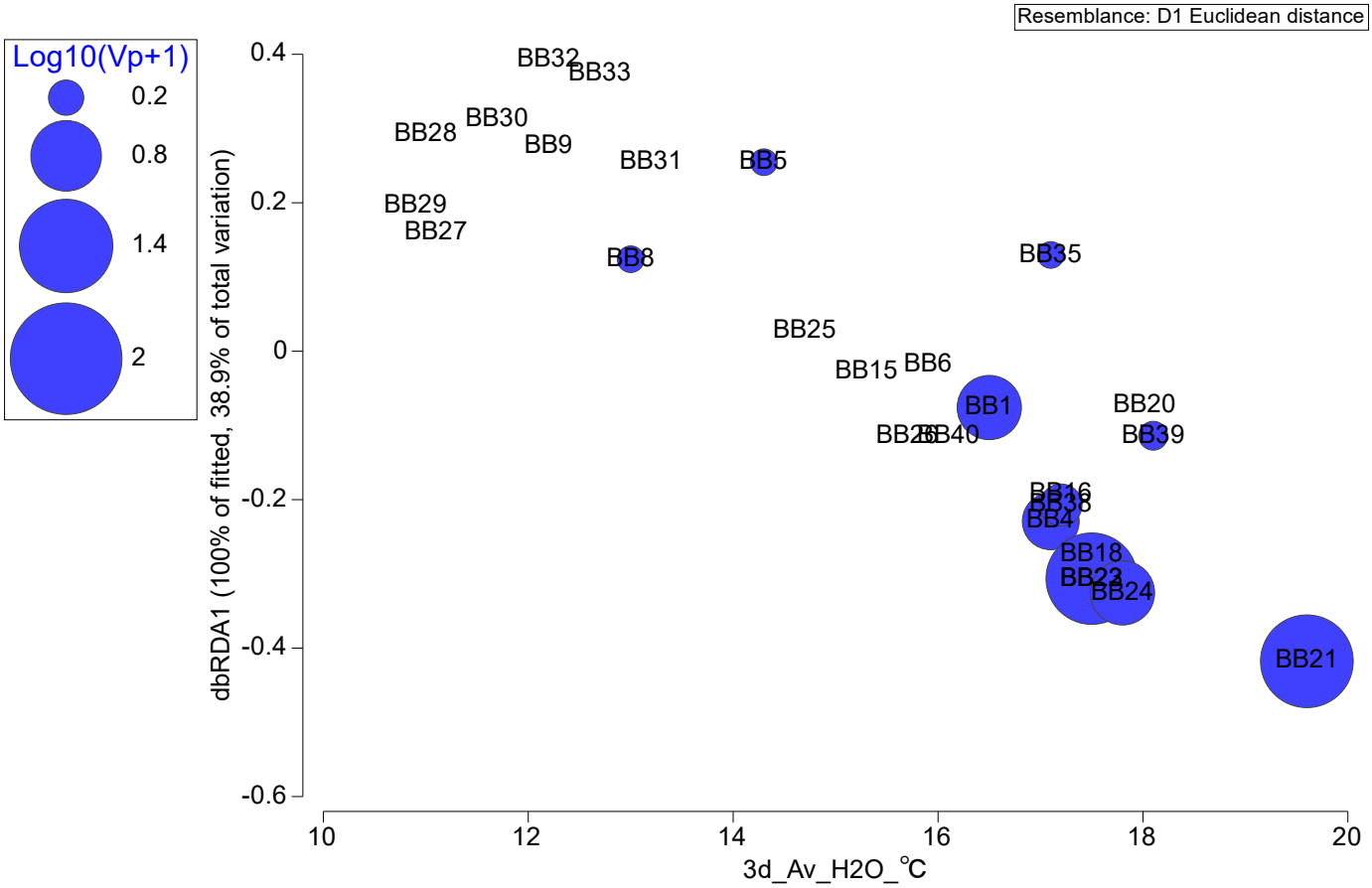

Figure 7: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with predictor variables 3-day average water temperature and 7-day rainfall. Bubbles on plot indicate level of *V. parahaemolyticus* (Vp) detected in mussels sampled from Boomer Bay.

The predictive model developed for *V. parahaemolyticus* in Boomer Bay was based on the 3-day average water temperature and 7-day rainfall prior to harvest:

$$y = -0.6016 + 0.0640x_0 - 0.0516x_1$$

Where:

y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = 3-day average water temperature prior to sampling (°C)
x_1 = 7-day rainfall prior to sampling (mm)
R^2 = 0.39

Conclusions

- *V. parahaemolyticus* was detected in Boomer Bay during all three summer/autumn sampling periods with a prevalence of 33-75%. No *V. parahaemolyticus* was detected during any of the winter/spring sampling periods.
- Levels of *V. parahaemolyticus* detected during the survey period were low; less than ≤23 MPN/g in oysters.
- *V. parahaemolyticus* carrying the *tdh* and/or *trh* genes, often associated with clinical strains, were only detected in two of the summer/autumn seasons with a prevalence of ≤13%.
- *V. parahaemolyticus* was not detected when the sampling water temperature was <12°C, the 3-day average water temperature preceding sampling was <13°C or the minimum water temperature 3-days prior to sampling was <8°C. Three-day average water temperature and 7-day rainfall preceding oyster harvest are good indicators for *V. parahaemolyticus* risk in Boomer Bay. The linear predictive model developed for this harvest area is:

$$y = -0.6016 + 0.0640x_0 - 0.0516x_1$$

  Where:
  y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
  x_0 = 3-day average water temperature prior to sampling (°C)
  x_1 = 7-day rainfall prior to sampling (mm)
  R^2 = 0.39

- The predictive models developed for *V. parahaemolyticus* risk in Boomer Bay and Boomer Bay East were both strongly driven by the harvest area water temperature. 30% of the explained variability in *V. parahaemolyticus* numbers was driven by the 3-day average water temperature in Boomer Bay, while 26% of the explained variability in *V. parahaemolyticus* numbers was driven by minimum water temperature 3-days prior to harvest in Boomer Bay East. Rainfall was associated with *V. parahaemolyticus* in Boomer Bay but not in Boomer Bay East. Whilst the two growing areas are connected, they do experience different environmental factors, and this may explain the variance in the models. Boomer Bay has higher flushing rates and tidal forces and is more exposed to wind than Boomer Bay East. It is also less impacted by rainfall (as shown by a smaller salinity range) but does have different catchment uses including more agricultural activities, potentially resulting in different run-off characteristics. It is unknown whether the association between rainfall and *V. parahaemolyticus* numbers in Boomer Bay is due to a run-off or could be a result of weather conditions that co-occur with rain (e.g., wind resulting in resuspended sediments). It should be noted that the data set collected for Boomer Bay was larger than for Boomer Bay East during the survey and hence more comprehensive.
- *V. vulnificus* was not detected in Boomer Bay during the survey period, although it was detected in Boomer Bay East in the third summer/autumn sampling period in one sample (0.3 MPN/g in oyster).

Appendix 8: *Vibrio* Survey Boomer Bay East, 2020-2022

Background

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Boomer Bay East (Figure 1).

Figure 1: Boomer Bay East harvest area. Orange pin indicates lease from which sampling occurred. Blue location marker indicates BOM weather station at Dunalley. The decimal degree (DD) coordinates of the Boomer Bay East lease is (-42.88334, 147.87497). Yellow star indicates lease in Boomer Bay (-42.85968, 147.87979). Scale bar = 2 km.

Methodology

Summary of survey design

Oysters were sampled over three summer/autumn (November to April) and two winter/spring (May to October) periods from January 2020 to end of April 2022. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data collected: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data collected: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity, water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation. Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station

number 94254; Dunalley - Stroud Point). Maximum river flow on the day of sampling was also accessed from the Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable, for the river identified in the harvest area's sanitary survey. For Boomer Bay East no river was identified. Rainfall and maximum daily air temperature were taken from the same BOM station for both Boomer Bay and Boomer Bay East samples, although sampling generally occurred on different days. Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 94254; Dunalley - Stroud Point).

Statistical analysis

Statistical analysis of biological and environmental data was done in Primer 7 + PERMANOVA (Primer-e). Correlation among variables was investigated using draftsman's plots. *V. parahaemolyticus* (MPN/g) data were $\log_{10}(X+1)$ transformed and used to create a Euclidean distance resemblance matrix. The environmental data was also transformed if not normally distributed: rainfall data for Boomer Bay East was square root transformed. The DISTLM (distance-based linear model) routine was used to investigate the relationship between the *V. parahaemolyticus* Euclidean distance resemblance matrix and the environmental (temperature, salinity, rainfall and river flow) predictor variables. The step-wise "selection procedure" was used with the Akaike Information Criterion corrected for small sample sizes (AICc) "selection criterion" and 9999 permutations. AICc is a mathematical method for evaluating how well a model fits the data it was generated from. The output of this analysis gives a statistical marginal test for each individual predictor variable considered alone, and the best overall solution. The best linear model was visually represented using dbRDA (distance-based redundancy analysis). The formula for the resulting predictive model was generated using the regression function in excel.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Salinity and rainfall data during the survey period are shown in Figure 2A. It should be noted that no sampling occurred between May and December 2020, therefore no environmental data was collected or presented during this period. The highest rainfall events occurred in the summer/autumn months. Figure 2B shows *V. parahaemolyticus* levels and sampling water temperature and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Temperature and *V. parahaemolyticus* numbers increased during the summer/autumn months.

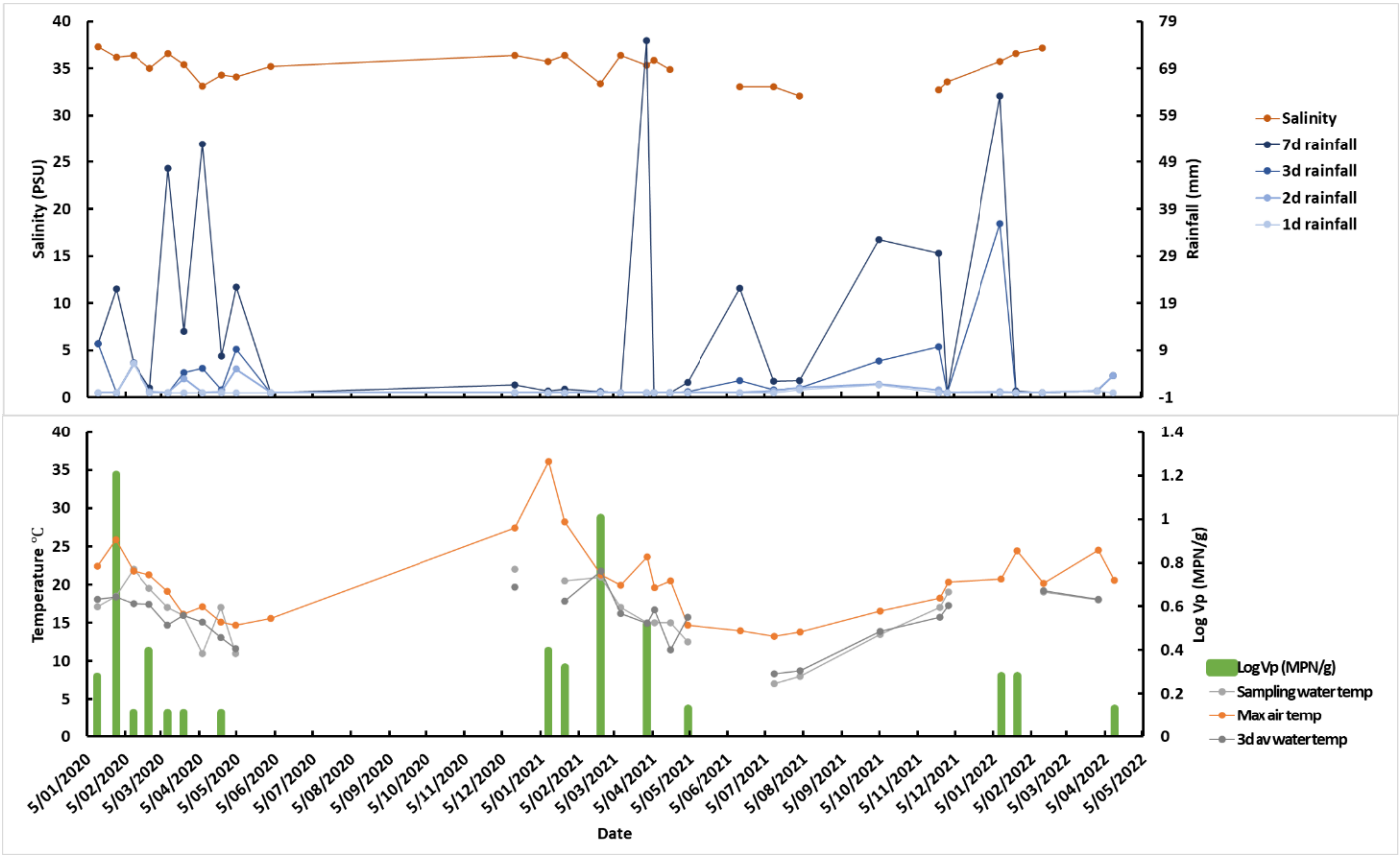

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Boomer Bay East. 2A shows water salinity at sampling and rainfall at Dunalley (1, 2, 3 and 7 days) prior to sampling. 2B shows levels of *V. parahaemolyticus* detected and are overlayed with sampling water temperature, 3-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence for Boomer Bay East broken down to the 5 seasons sampled during the survey. It should be noted that winter year 1 may not be truly representative of that season as only two samples were collected during the period: one in May and the other in June 2020 (Figure 2). Sampling resumed in December 2020 for this growing area. *V. parahaemolyticus* prevalence was higher in the summer/autumn sampling periods (43-88%). A lower prevalence of *V. parahaemolyticus* (20%) was still detected in the winter/spring sampling period of 2021. No *V. parahaemolyticus* strains containing the *trh* gene were detected during the survey period. *V. parahaemolyticus* strains containing the pathogenicity associated *tdh* gene was only detected in the third summer/autumn sampling period.

V. vulnificus was also only detected in the third summer/autumn sampling period with a low prevalence of 14%.

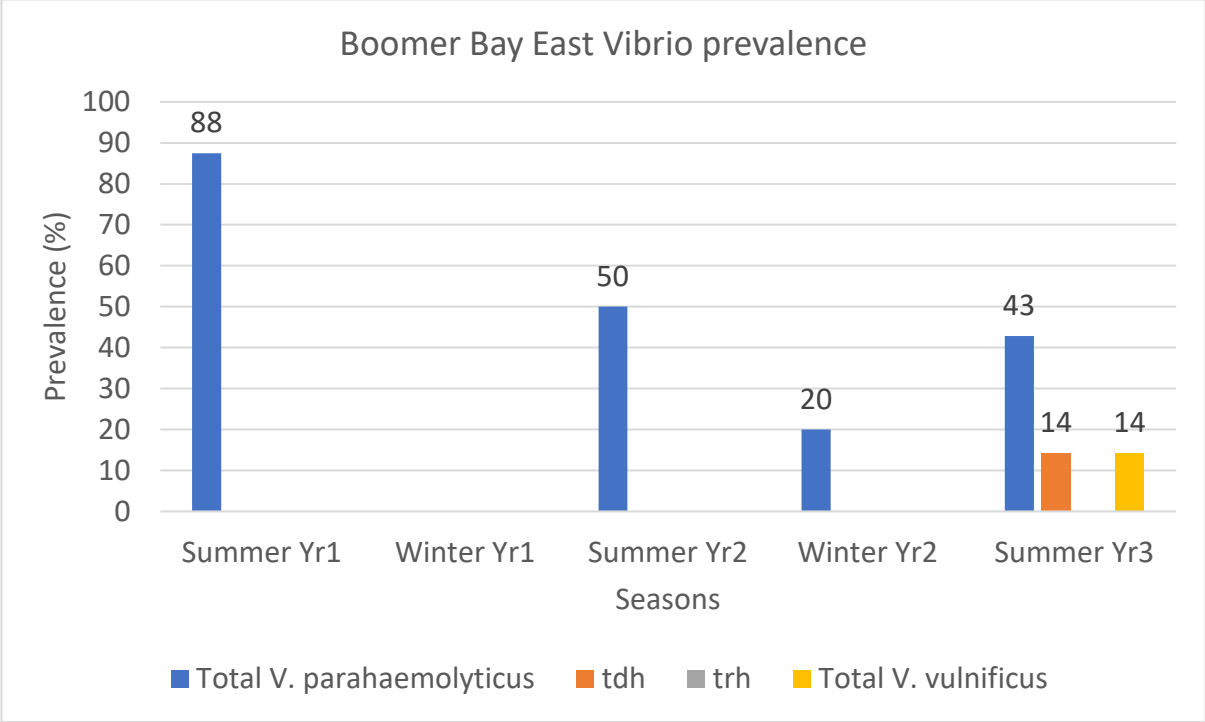

Figure 3: Seasonal *Vibrio* prevalence during the survey period.

V. parahaemolyticus was not detected when sampling water temperature was <15°C, when 3-day average water temperature prior to sampling was <13°C or when minimum water temperature 3-days prior to sampling was <8°C. *V. vulnificus* was detected in one sample when the 3-d average water temperature was 16°C and salinity was 33 PSU.

Of the 25 samples collected during the survey period, n=17 were collected when oysters were below the water; the remaining samples had no recorded information on the sample submission form. Most of the samples were collected at low, low incoming or low outgoing tidal (n=15/25) stages. No relationship could be deduced with tidal stage and *V. parahaemolyticus* (Figure 4).

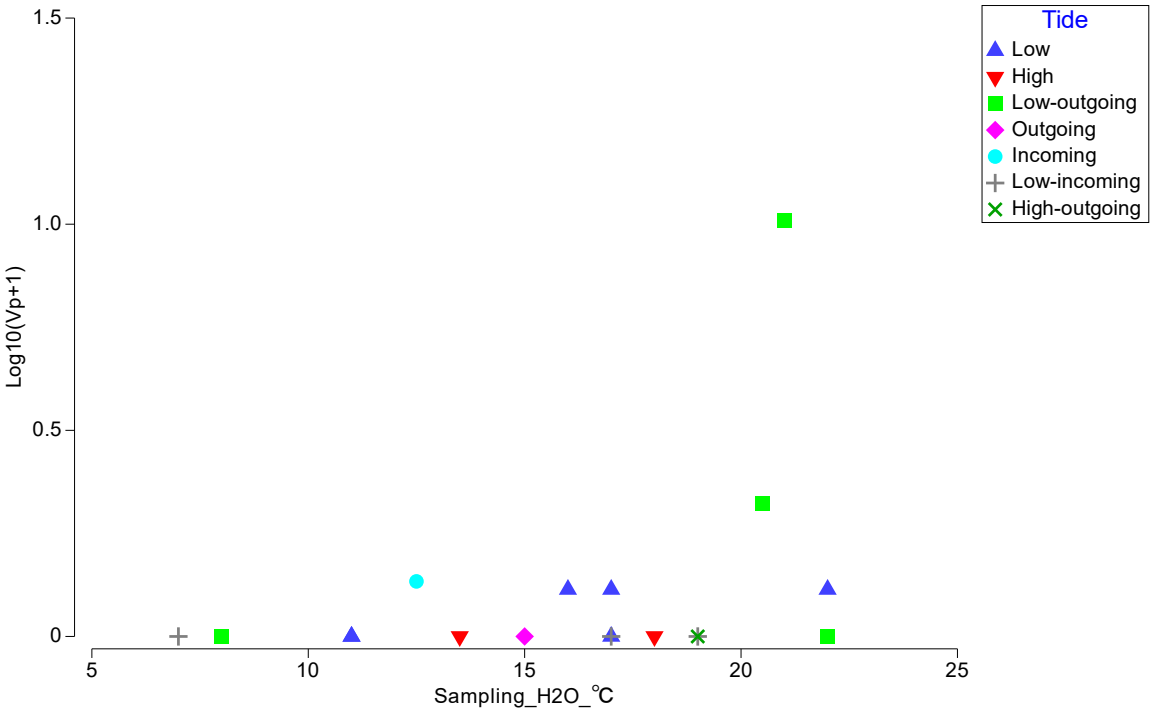

Figure 4: Scatter plot of *V. parahaemolyticus* versus sampling temperature identified by tidal stage of collection.

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data collected during the survey, aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of temperatures were recorded in the growing area over the period of the survey. Salinity in the growing area was stable and ranged from 32-37 PSU. *V. parahaemolyticus* numbers where low, with a maximum detection of 9.2 MPN/g. *V. vulnificus* numbers were even lower at <1 MPN/g.

Table 1: Summary table of environmental parameter over the summer/autumn and winter/spring survey periods.

	Summer (n=18)					**Winter (n=7)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	11.0	22.0	17.8	3.0	15	7.0	13.5	10.4	2.8	5
Sampling air temp	16.0	19.0	17.5	2.1	2	4.0	15.0	8.3	5.9	3
3-day min H_2O temp	8.5	20.5	14.2	3.2	15	4.0	11.5	8.0	2.8	5
3-day max H_2O temp	16.5	28.5	21.7	3.7	15	9.5	18.5	13.7	4.0	5
3-day Av H_2O temp	13.1	21.8	16.9	2.2	15	8.3	15.7	11.6	3.2	5
1-day max air temp	15.1	28.2	21.0	3.6	18	13.2	16.5	14.6	1.1	7
Salinity (PSU)										
Sampling H_2O	32.7	37.1	35.3	1.4	16	32.1	35.2	33.5	1.2	5
Rainfall (mm)										
1-day	0	6.2	0.4	1.5	18	0	1.6	0.3	0.6	7
2-days	0	6.2	0.8	1.7	18	0	5.0	1.2	1.8	7
3-days	0	35.8	3.7	8.5	18	0	9.2	2.9	3.6	7
7-days	0	74.9	16.8	25.1	18	0	32.4	12.0	13.2	7

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods.

***Vibrio* enumeration (MPN/g)**						
Vibrio	Summer (n=18)			Winter (n=7)		
	Min	Max	Positive (n=)	Min	Max	Positive (n=)
Total *V. parahaemolyticus*	0	9.2	10	0	0.36	1
tdh	0	0.36	1	0	0	0
trh	0	0	0	0	0	0
Total *V. vulnificus*	0	0.3	1	0	0	0

Statistical analysis

Temperature

The temperature predictor variables were all correlated (R>0.51) with one another (Figure 5). The 3-day average water temperature and sampling water temperature or minimum water temperature 3 days prior to sampling were the most correlated (R=0.89). *V. parahaemolyticus* numbers were most correlated with the minimum water temperature 3 days prior to shellfish sampling (R=0.51).

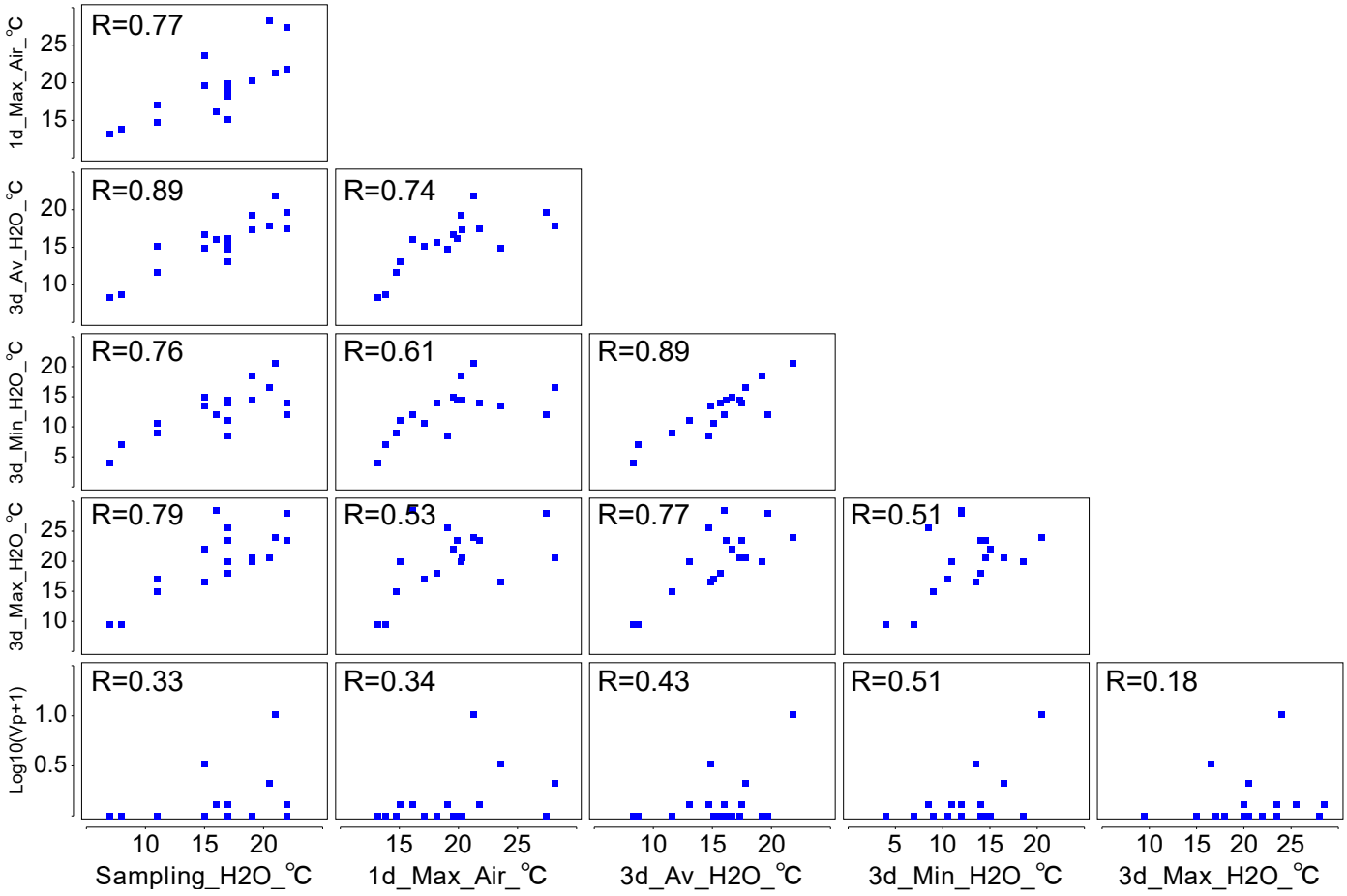

Figure 5: Draftsman's plots of temperature variables and *V. parahaemolyticus*.

Rainfall and salinity

Salinity was poorly correlated with rainfall (Figure 6). Salinity was somewhat negatively correlated with 3-day rainfall. Rainfall was also somewhat correlated with one another, with the highest correlation observed between 2- and 3-day rainfall. Poor correlation was observed between *V. parahaemolyticus* numbers and salinity or rainfall.

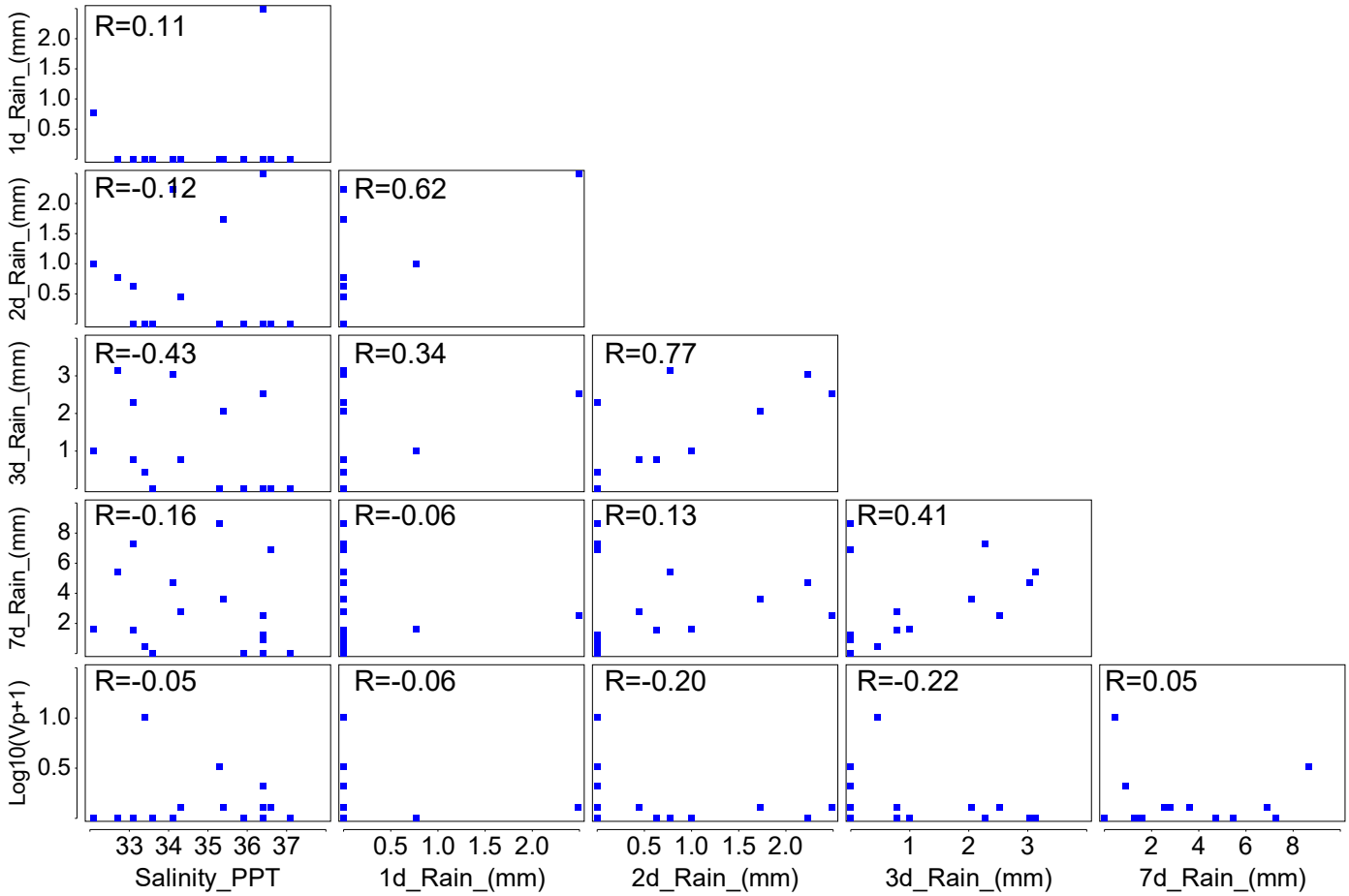

Figure 6: Draftsman's plots of rainfall and salinity variables and *V. parahaemolyticus*.

Linear model generation

The marginal statistical tests in DISTLM only showed a significant relationship between minimum water temperature 3-days prior to sampling (Pseudo-F=5.4, P=0.042) and *V. parahaemolyticus* numbers, individually explaining 26% of the explained variability. In the most accurate model selection this predictor variable also described 100% of the explained variability in the fitted model, 26% of the total variability in the data cloud (AICc=-46.46 R^2=0.26) (Figure 7).

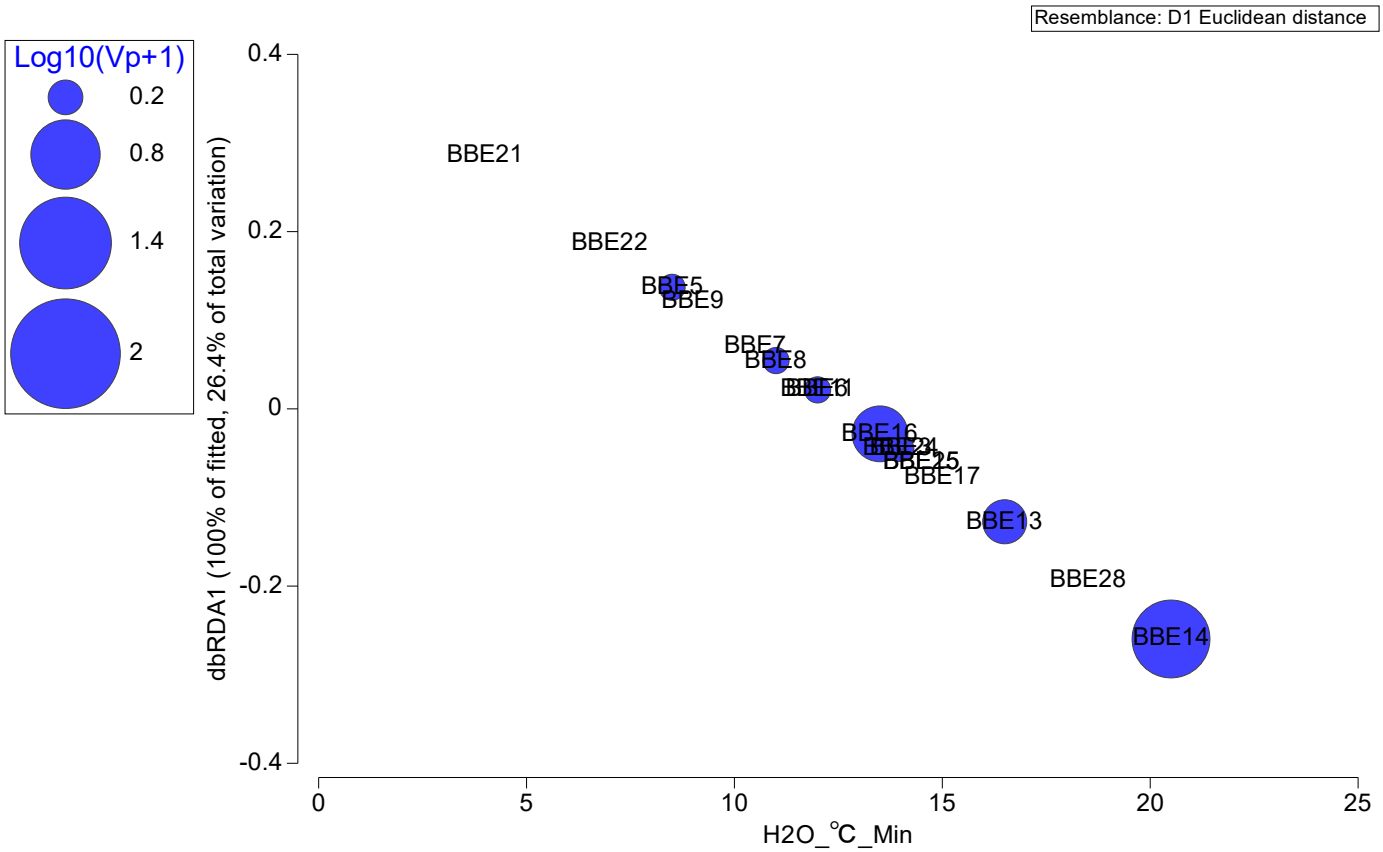

Figure 7: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with the predictor variable of minimum water temperature 3-day prior to sampling. Bubbles on plot indicate level of *V. parahaemolyticus* (Vp) detected in oysters from Boomer Bay East.

The predictive model developed for *V. parahaemolyticus* in Boomer Bay East was based on the minimum water temperature 3-days preceding shellfish harvest:

$$y = 0.0331x - 0.2827$$

Where:

y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)

x = minimum water temperature 3-days prior to sampling (°C)

R^2 = 0.26

Conclusions

- *V. parahaemolyticus* was detected in Boomer Bay East in all summer/autumn sampling seasons between 2020 and 2022, with a prevalence of 43-88%.
- *V. parahaemolyticus* was detected in Boomer Bay East in the second winter/spring sampling season in 2021 with a prevalence of 20%
- Levels of V. *parahaemolyticus* detected during the survey period were very low; <10 MPN/g in oysters.
- No *V. parahaemolyticus* strains carrying the *trh* gene were detected during the survey and *V. parahaemolyticus* carrying the *tdh* gene was only detected in the third summer/autumn sampling season with a prevalence of 14%.
- *V. parahaemolyticus* was not detected when sampling water temperature was <15°C, when 3-day average water temperature prior to sampling was <13°C or when minimum water temperature 3-days prior to sampling was <8°C. Minimum water temperature 3-days preceding harvest is a good indicator of *V. parahaemolyticus* risk in Boomer Bay East. The linear predictive model developed for this harvest area is:

  $$y = 0.0331x - 0.2827$$

  Where:
  y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
  x = minimum water temperature 3-days prior to sampling (°C)
  R^2 = 0.26
- The predictive models developed for *V. parahaemolyticus* risk in Boomer Bay and Boomer Bay East were both strongly driven by the harvest area water temperature. 30% of the explained variability in *V. parahaemolyticus* numbers was driven by the 3-day average water temperature in Boomer Bay, while 26% of the explained variability in *V. parahaemolyticus* numbers was driven by minimum water temperature 3-days prior to harvest in Boomer Bay East. The different models may be explained by the impact of different environmental factors in these adjacent harvest areas. Boomer Bay has higher flushing rates and tidal forces and is more exposed to wind than Boomer Bay East. It is also less impacted by rainfall (as shown by a smaller salinity range) but does have different catchment uses including more agricultural activities, potentially resulting in different run-off characteristics. It is unknown whether the association between rainfall and *V. parahaemolyticus* numbers in Boomer Bay is due to a run-off or could be a result of weather conditions that co-occur with rain (e.g., wind resulting in resuspended sediments). It should be noted that the data set collected for Boomer Bay was larger than for Boomer Bay East during the survey and hence more comprehensive.
- *V. vulnificus* was only detected during the third summer/autumn sampling period with a prevalence of 14% and levels <1 MPN/g of oysters.

Appendix 9: *Vibrio* Survey Pitt Water, 2020-2022

Background

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Pitt Water (Figure 1).

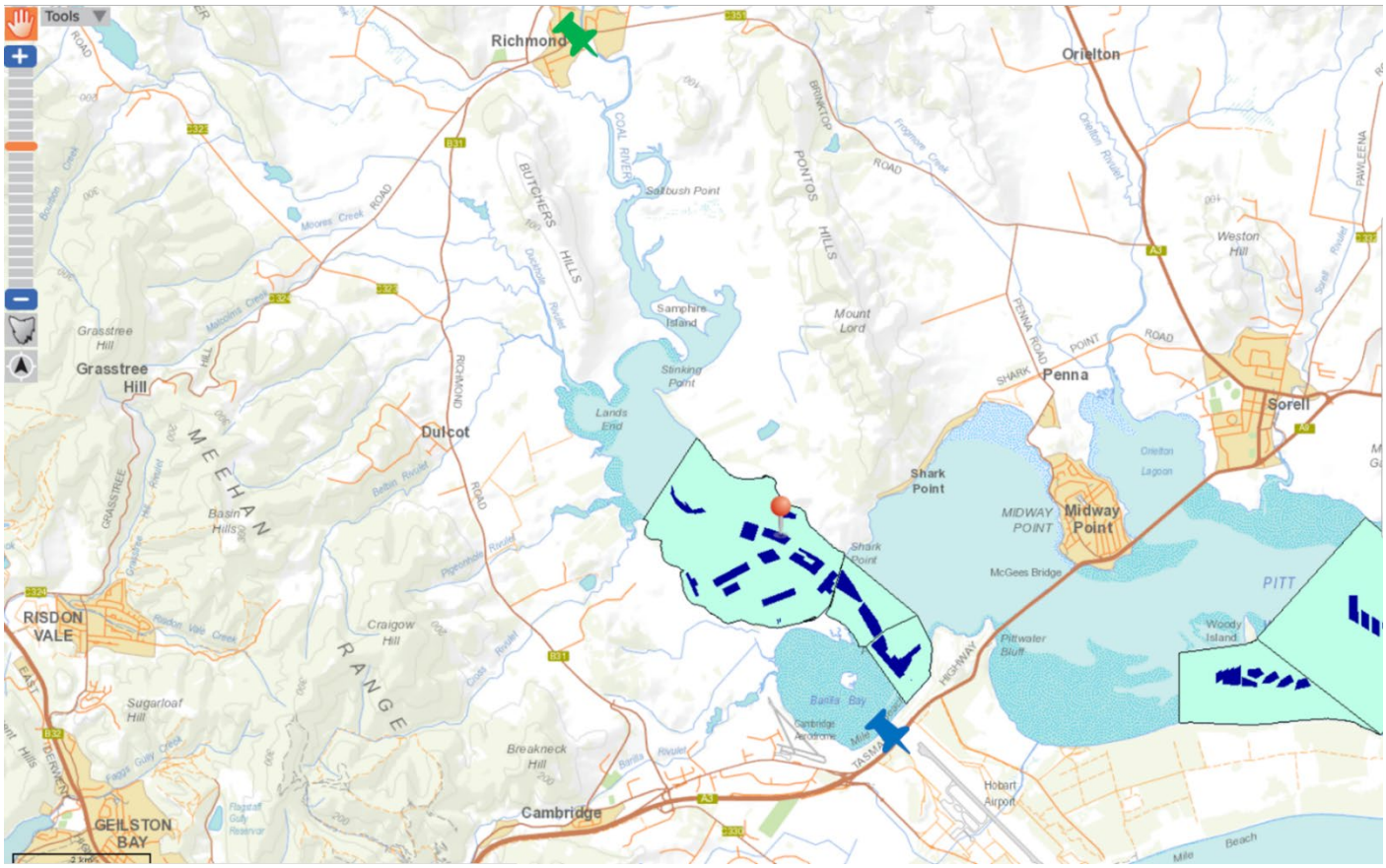

Figure 1: Pitt Water harvest area. Orange pin indicates lease from which sampling occurred during the survey period. Green pin indicates Coal River flow station at Richmond. Blue pin indicates BOM weather station at Hobart Airport West. Scale bar = 2 km.

Methodology

Summary of survey design

Oysters were sampled over three summer/autumn (November to April) and two winter/spring (May to October) periods from January 2020 to end of April 2022. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data inputs: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data inputs: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity, water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation. Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station number 94008; Hobart Airport West). Maximum river flow on the day of sampling was also accessed from the Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable, for the river identified in the harvest area's sanitary survey. For Pitt Water this was the Coal River at Richmond.

Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 94008; Hobart Airport West).

Statistical analysis

Statistical analysis of biological and environmental data was done in Primer 7 + PERMANOVA (Primer-e). Correlation among variables was investigated using draftsman's plots. *V. parahaemolyticus* (MPN/g) data were $\log_{10}(X+1)$ transformed and used to create a Euclidean distance resemblance matrix. The environmental data was also transformed if not normally distributed: rainfall and the river flow data for Pitt Water were fourth root transformed. The DISTLM (distance-based linear model) routine was used to investigate the relationship between the *V. parahaemolyticus* Euclidean distance resemblance matrix and the environmental (temperature, salinity, rainfall and river flow) predictor variables. The step-wise "selection procedure" was used with the Akaike Information Criterion corrected for small sample sizes (AICc) "selection criterion" and 9999 permutations. AICc is a mathematical method for evaluating how well a model fits the data it was generated from. The output of this analysis gives a statistical marginal test for each individual predictor variable considered alone, and the best overall solution. The best linear model was visually represented using dbRDA (distance-based redundancy analysis). The formula for the resulting predictive model was generated using the regression function in excel.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Salinity and rainfall data during the survey period are shown in Figure 2A. The highest rainfall events were recorded in summer months and usually corresponded to decreased growing water salinity and increased river flow of the Coal River (Figure 2B). Figure 2C shows *V. parahaemolyticus* levels, sampling water temperature and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Temperature and *V. parahaemolyticus* numbers increased during summer/autumns months. Salinity in Pitt Water was variable (21-37 PSU).

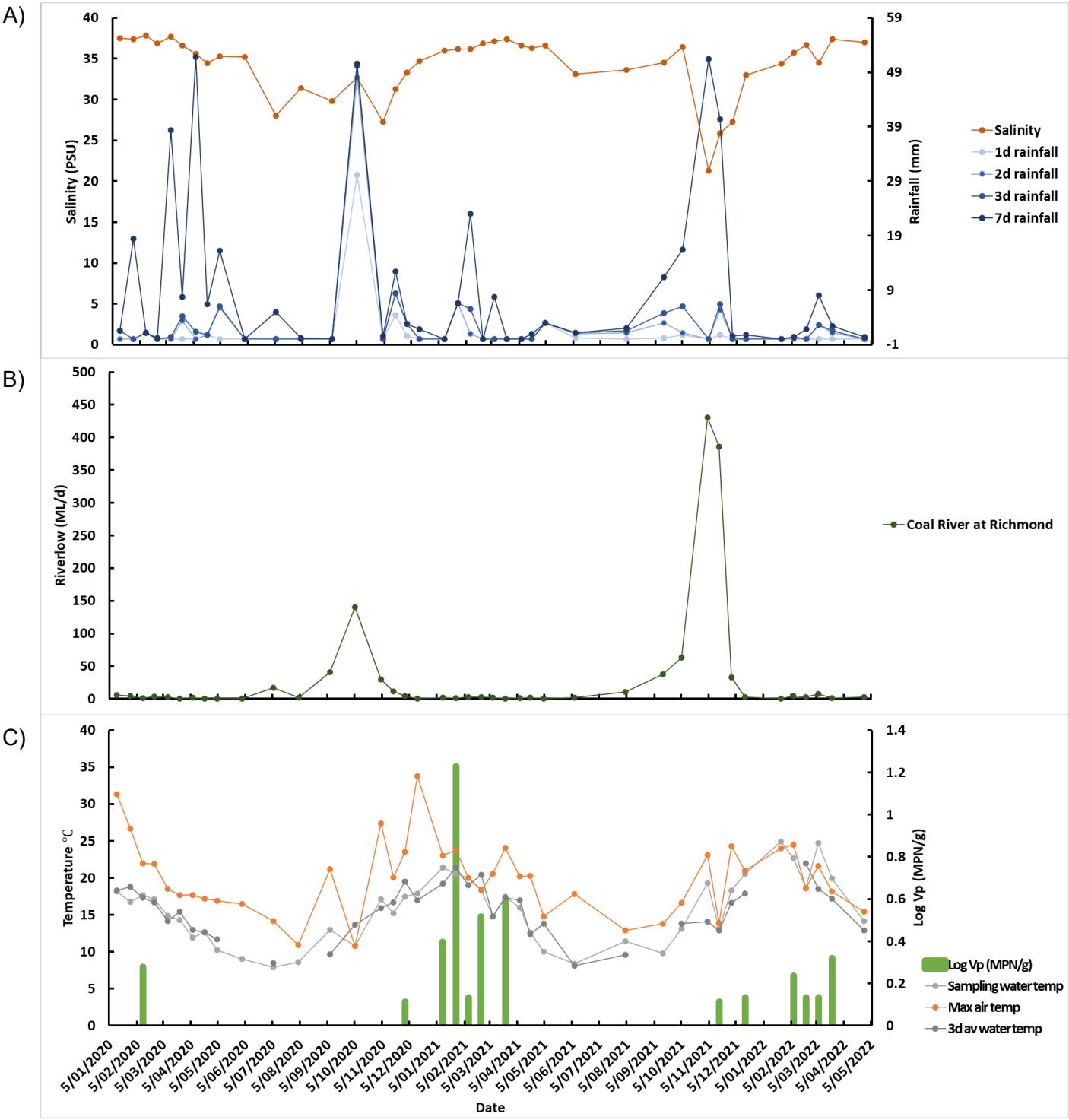

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Pitt Water. 2A shows water salinity at sampling and rainfall at Hobart airport (1, 2, 3 and 7 days) prior to sampling. 2B shows maximum river flow 24 hours prior to sampling. 2C shows levels of *V. parahaemolyticus* detected, overlayed with sampling water temperature, three-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence for Pitt Water broken down to the 5 seasons sampled during the survey. *V. parahaemolyticus* was only detected during summer months, with a prevalence of 13-60%. No *V. parahaemolyticus* strains carrying the pathogenicity associated *tdh* and *trh* genes were detected during the survey period. *V. vulnificus* was only detected in the summer/autumn period of year 1 and 3 with a prevalence of 13 and 30%, respectively.

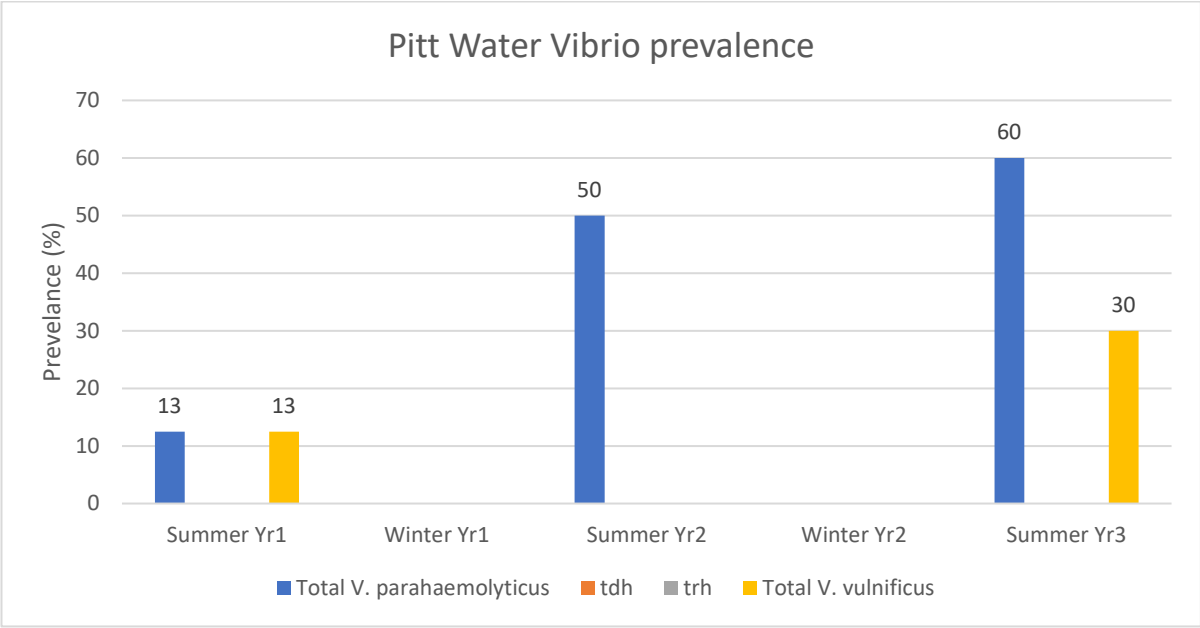

Figure 3: Seasonal *Vibrio* prevalence during the survey period.

V. parahaemolyticus was not detected when sampling water temperature was <13°C, 3-day average water temperature before sampling were <12°C and minimum water temperature 3 days before sampling was <6°C.

Of the 40 samples collected during the survey period, n=27 were taken below from the water, n=10 were taken from above the water and n=3 had no information recorded. Of the 13 samples which tested positive for *V. parahaemolyticus* in the summer/autumn season, n=9 were collected from below the water and n=4 were collected from above the water. No clear relationship between tidal stage and *V. parahaemolyticus* levels could be deducted (Figure 4).

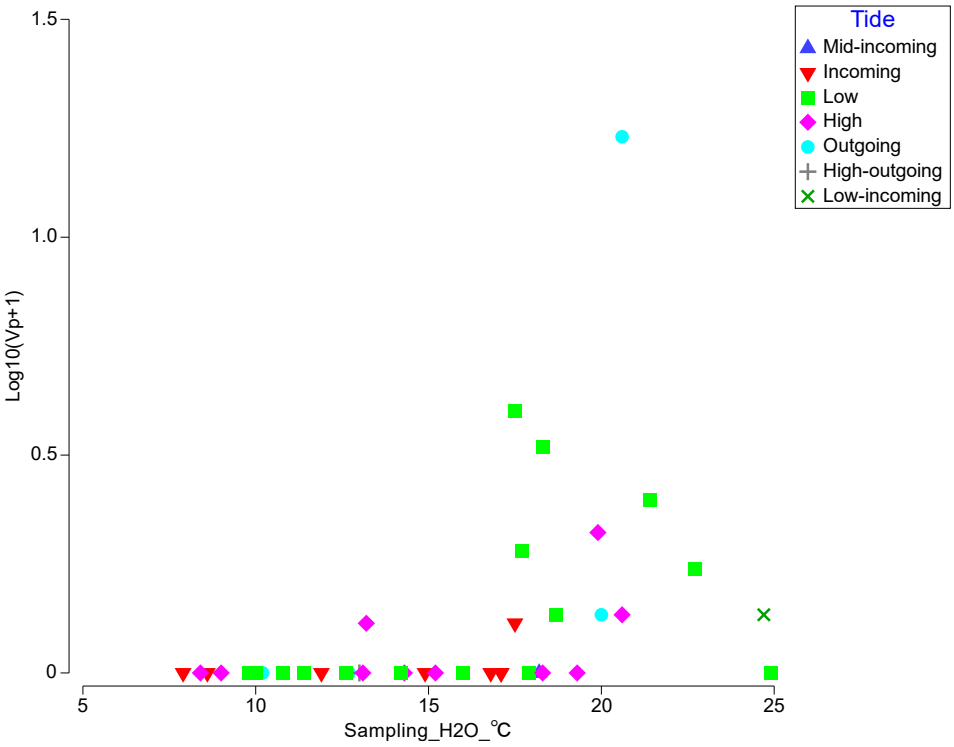

Figure 4: Scatter plot of *V. parahaemolyticus* versus sampling temperature identified by tidal stage of collection.

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data during the survey aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of environmental conditions were recorded in the growing area over the period of the survey. *V. parahaemolyticus* numbers where low at ≤16 MPN/g oyster. *V. vulnificus* was detected in four samples at very low levels of <1 MPN/g. Corresponding water salinity in for these positive samples ranged from 26 to 37 PSU.

Table 1: Summary table of environmental parameters over the summer/autumn & winter/spring survey periods.

	Summer (n=29)					**Winter (n=11)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	11.9	24.9	17.7	3.4	29	7.9	13.1	10.2	1.8	11
Sampling air temp	6.0	19.3	14.8	3.7	14	9.4	10.3	9.9	0.5	3
3-day min H_2O temp	6.0	20.5	11.7	3.3	27	0	1.3	6.8	3.5	7
3-day max H_2O temp	15.5	30.5	21.8	3.7	27	10.0	25.0	16.5	5.2	8
3-day Av H_2O temp	12.4	22.0	16.8	2.7	27	8.1	13.8	11.1	2.4	8
1-day max air temp	13.9	33.8	21.8	4.4	29	10.8	21.2	15.1	3.0	11
Salinity (PSU)										
Sampling H_2O	21.3	37.8	34.5	4.1	29	28.1	36.6	33.3	2.7	11
Rainfall (mm)										
1-day	0	6.6	0.5	1.4	29	0	30.	3.1	9.0	11
2-days	0	8.4	1.2	2.2	29	0	48.0	5.7	14.1	11
3-days	0	8.4	1.5	2.4	29	0	50.2	6.6	14.7	11
7-days	0	51.8	9.7	15.8	29	0	50.6	9.6	15.0	11
River flow (ML/d)										
Coal River	0.3	430.6	32.7	104.52	29	0.2	14.0	28.6	42.6	11

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods

***Vibrio* enumeration (MPN/g)**						
Vibrio	**Summer (n=29)**			**Winter (n=11)**		
	Min	**Max**	**Positive (n=)**	**Min**	**Max**	**Positive (n=)**
Total *V. parahaemolyticus*	0	16	13	0	0	0
tdh	0	0	0	0	0	0
trh	0	0	0	0	0	0
Total *V. vulnificus*	0	0.73	4	0	0	0

Statistical analysis

Temperature

All the temperature predictors were positively correlated with the water temperature predictor variables generally showing higher correlation amongst one another (Figure 5). The 3-day average water temperature preceding sampling showed the highest correlation with the minimum water temperature 3 days before sampling (R=0.86), followed by water sampling temperature (R=0.84). *V. parahaemolyticus* numbers showed the highest correlation with both average and minimum water temperature 3-days before sampling (R=0.52).

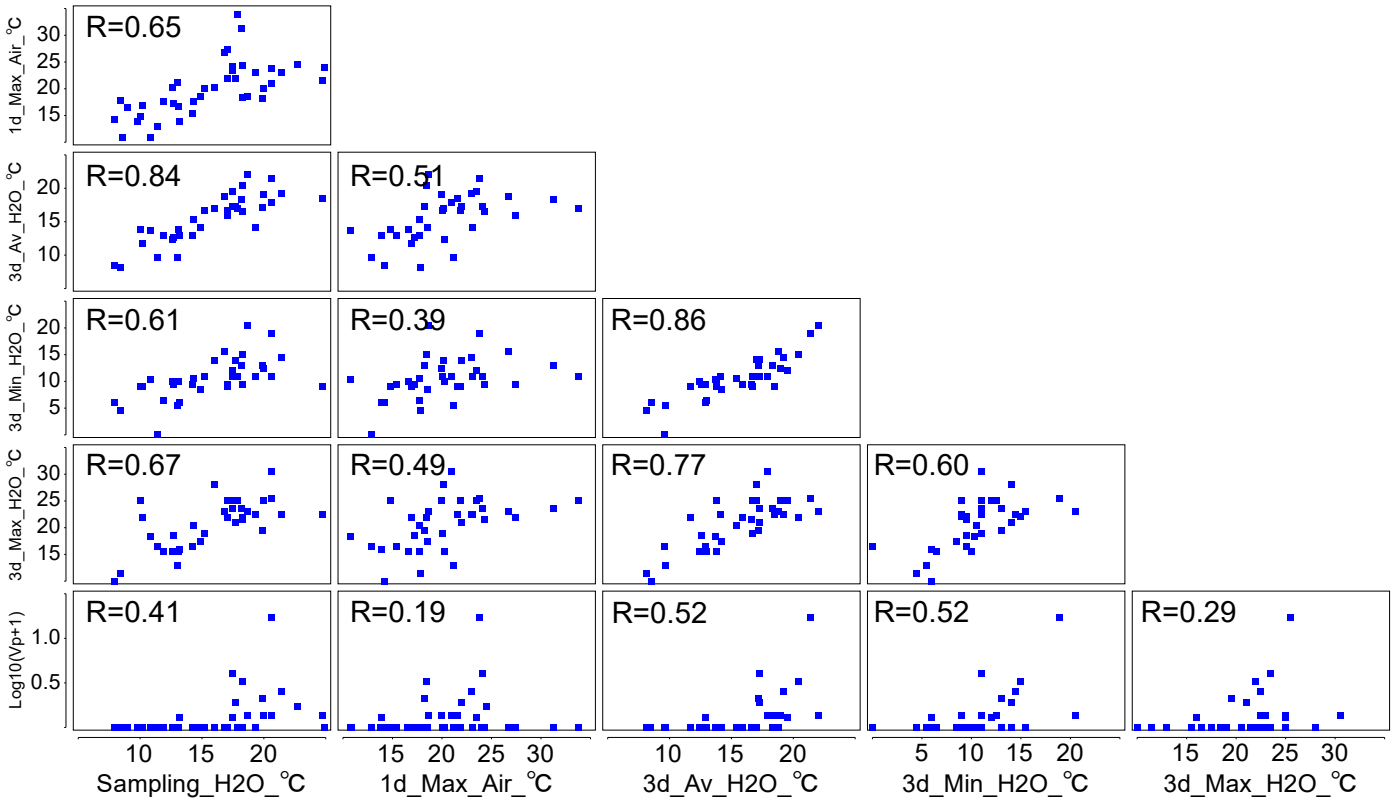

Figure 5: Draftsman's plots of temperature variables and *V. parahaemolyticus*.

Rainfall

Rainfall 1 to 7 days prior to oyster sampling were positively correlated (Figure 6). The highest correlations were observed between 2- and 3-days rainfall (R=0.91), followed by 1- and 2-days rainfall (R=0.80). *V. parahaemolyticus* numbers were poorly correlated with rainfall, although the highest correlation was observed with 1-day rainfall (R=0.20).

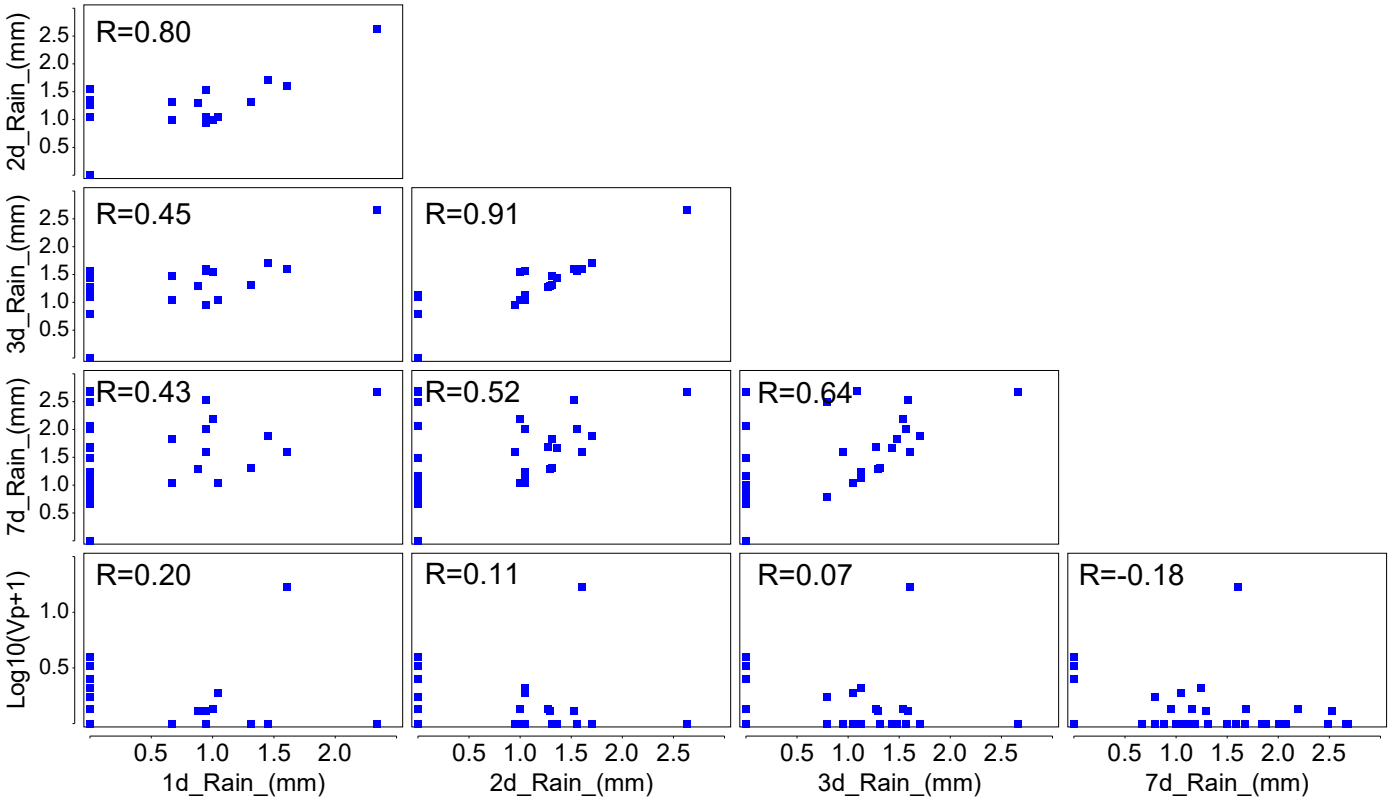

Figure 6: Draftsman's plots of rainfall variables and *V. parahaemolyticus*. Rainfall data was fourth root transformed.

Salinity and river flow

Maximum river flow of the Coal River at Richmond and salinity of the Pitt Water harvest area were negatively correlated (Figure 7). *V. parahaemolyticus* numbers were positively correlated with harvest water salinity (R=0.25) and negatively correlation with river flow (R=-0.20), although these correlations were both low.

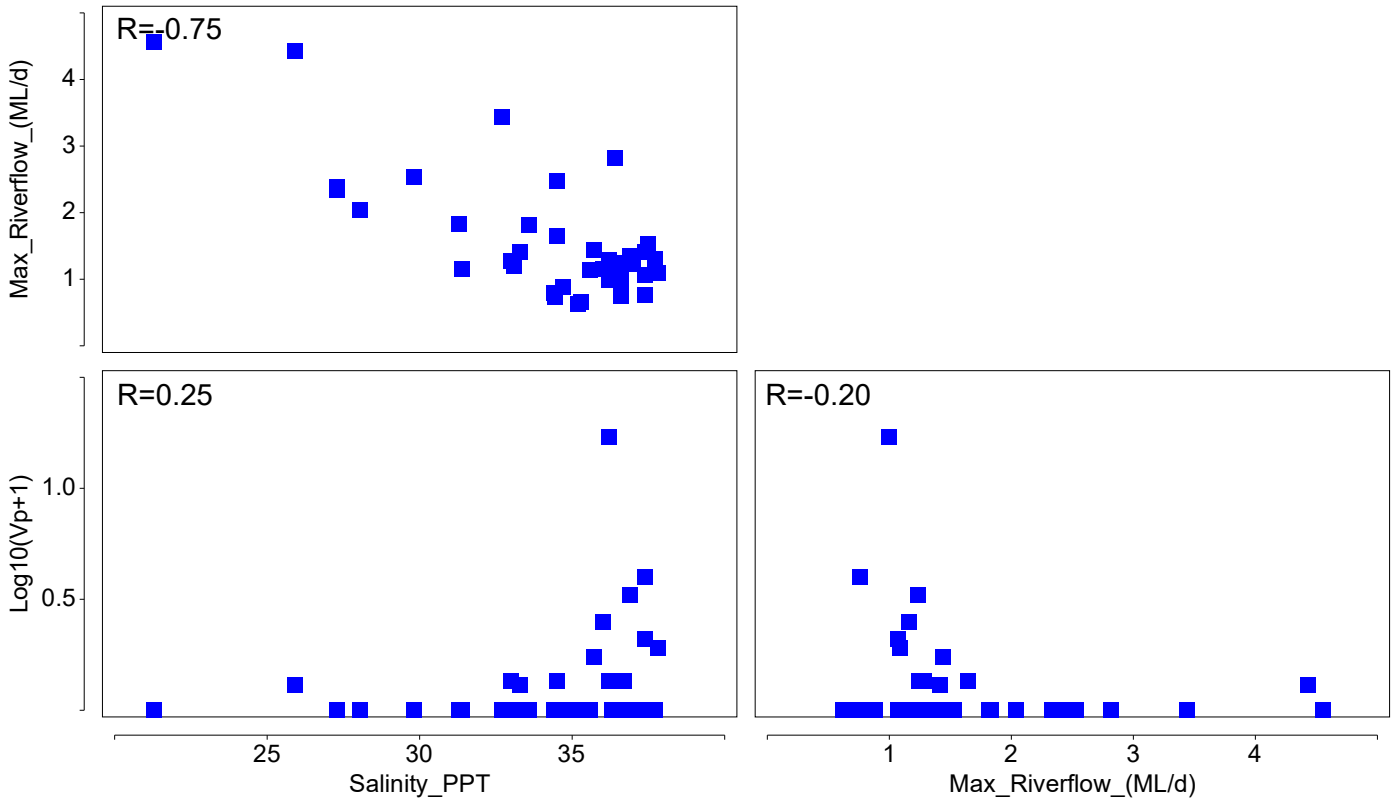

Figure 7: Draftsman's plots of salinity, river flow and *V. parahaemolyticus* variables. River flow data was fourth root transformed.

Linear predictive model generation

The marginal statistical tests in DISTLM showed significant relationships of sampling water temperature (Pseudo-F=8.4, P=0.005), 3-day average water temperature prior to sampling (Pseudo-F=12.0, P<0.001) and minimum water temperature 3 days prior to sampling (Pseudo-F=12.2, P=0.002) with *V. parahaemolyticus* numbers, individually explaining 20%, 27% and 27% of the explained variability. In the most accurate model selection minimum water temperature 3-days prior to sampling described 100% of the explained variability in the fitted model, 27% of the total variability in the data cloud (AICc=-105.15 R^2=0.27) (Figure 8).

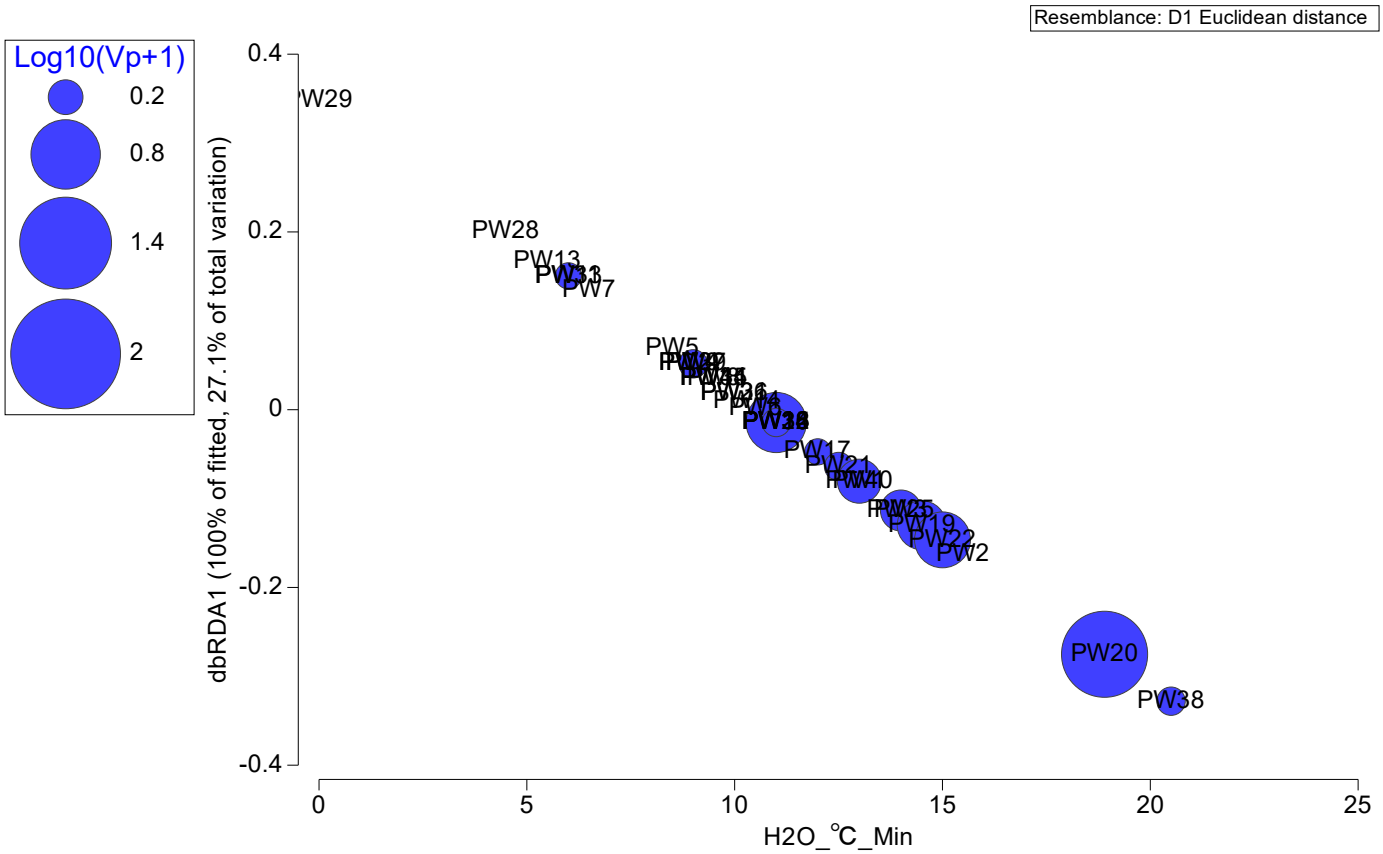

Figure 8: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with the predictor variable minimum water temperature 3 days prior to sampling. Bubbles on plot indicate level of *V. parahaemolyticus* (Vp) detected in oysters from Pitt Water.

The predictive model developed for *V. parahaemolyticus* in Pitt Water for oysters was based on the minimum water temperature 3 days prior to harvest:

$$y = -0.2312 + 0.0330x_0$$

Where:

y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)

x_0 = minimum water temperature 3 days prior to sampling (°C)

R^2 = 0.27

Conclusions

- *V. parahaemolyticus* was detected in Pitt Water only in the summer/autumn sampling seasons between 2020 and 2022, with a prevalence ranging from 13-60%.
- Levels of *V. parahaemolyticus* detected during the survey period were low; ≤16 MPN/g of oysters.
- No *V. parahaemolyticus* carrying the *tdh* and/or the *trh* genes were detected during the survey period.
- The sampling water temperature, average and minimum water temperature 3 days preceding sampling were good indicators of *V. parahaemolyticus* risk. *V. parahaemolyticus* was not detected when sampling water temperature was <13°C, 3-day average water temperature before sampling were <12°C and minimum water temperature 3 days before sampling was <6°C. These significant temperature predictors were correlated. The best predictive model for *V. parahaemolyticus* was based on the minimum water temperature 3 days prior to sampling.

$$y = -0.2312 + 0.0330x_0$$

Where:
y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = minimum water temperature 3 days prior to sampling (°C)
R^2 = 0.27

- *V. vulnificus* was only detected in the first and third summer/autumn sampling seasons, with a prevalence of 13% and 30% respectively. Levels detected were low at < 1 MPN/g. Salinity of sampling water temperature associated with these positive samples ranged from 22-37 PSU and 13-20°C, respectively. The average 3-day water temperature prior to sampling for these positives ranged from 13-19°C.

Appendix 10: *Vibrio* Survey Pipe Clay Lagoon, 2020-2022

Background

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Pipe Clay Lagoon (Figure 1).

Figure 1: Pipe Clay Lagoon harvest area. Orange pin indicates lease from which sampling occurred. Blue pin indicates BOM weather station at Hobart Airport West. Scale bar = 5 km.

Methodology

Summary of survey design

Oysters were sampled over three summer/autumn (November to April) and two winter/spring (May to October) periods from January 2020 to end of April 2022. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data collected: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data collected: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity, water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation. Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station number 94008; Hobart Airport West). Maximum river flow on the day of sampling was also accessed from the Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable,

for the river identified in the harvest areas' sanitary survey. No river input was identified for Pipe Clay Lagoon from the sanitary survey. Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 94008; Hobart Airport West).

Statistical analysis

Statistical analysis of biological and environmental data was done in Primer 7 + PERMANOVA (Primer-e). Correlation among variables was investigated using draftsman's plots. *V. parahaemolyticus* (MPN/g) data were $\log_{10}(X+1)$ transformed and used to create a Euclidean distance resemblance matrix. The environmental data was also transformed if not normally distributed: rainfall data for Pipe Clay Lagoon was fourth root transformed. The DISTLM (distance-based linear model) routine was used to investigate the relationship between the *V. parahaemolyticus* Euclidean distance resemblance matrix and the environmental (temperature, salinity and rainfall) predictor variables. The step-wise "selection procedure" was used with the Akaike Information Criterion corrected for small sample sizes (AICc) "selection criterion" and 9999 permutations. AICc is a mathematical method for evaluating how well a model fits the data it was generated from. The output of this analysis gives a statistical marginal test for each individual predictor variable considered alone, and the best overall solution. The best linear model was visually represented using dbRDA (distance-based redundancy analysis). The formula for the resulting predictive model was generated using the regression function in excel.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Salinity and rainfall data during the survey period are shown in Figure 2A. The highest rainfall events were recorded in spring, summer and autumn months. Figure 2B shows *V. parahaemolyticus* levels, sampling water temperature and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Temperature and *V. parahaemolyticus* numbers increased during summer/autumns months. Salinity in Pipe Clay Lagoon was relatively constant (33-37 PSU).

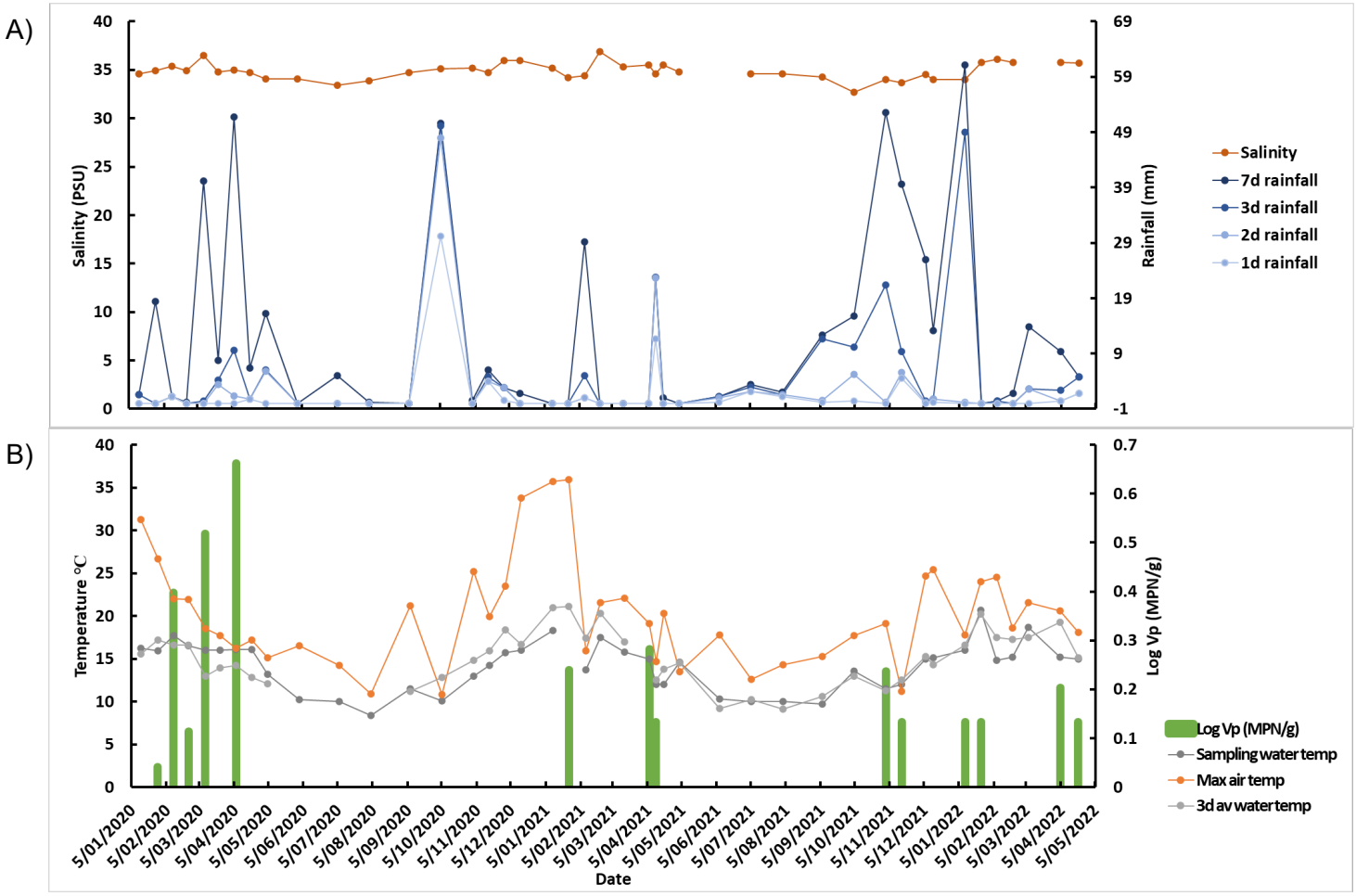

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Pipe Clay Lagoon. 2A shows water salinity at sampling and rainfall at Hobart Airport West (1, 2, 3 and 7 days) prior to sampling. 2B shows levels of *V. parahaemolyticus* detected, overlayed with sampling water temperature, three-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence for Pipe Clay Lagoon broken down to the 5 seasons sampled during the survey. *V. parahaemolyticus* was only detected in the summer/autumn sampling seasons between 2020-22 with a high prevalence ranging from 25-63%. *V. parahaemolyticus* containing the pathogenicity associated *tdh* and/or *trh* were detected in the first two summer/autumn sampling periods, with a prevalence ranging from 8-13%. *V. vulnificus* was detected in the first and third summer/autumn periods with a prevalence of 9-13%.

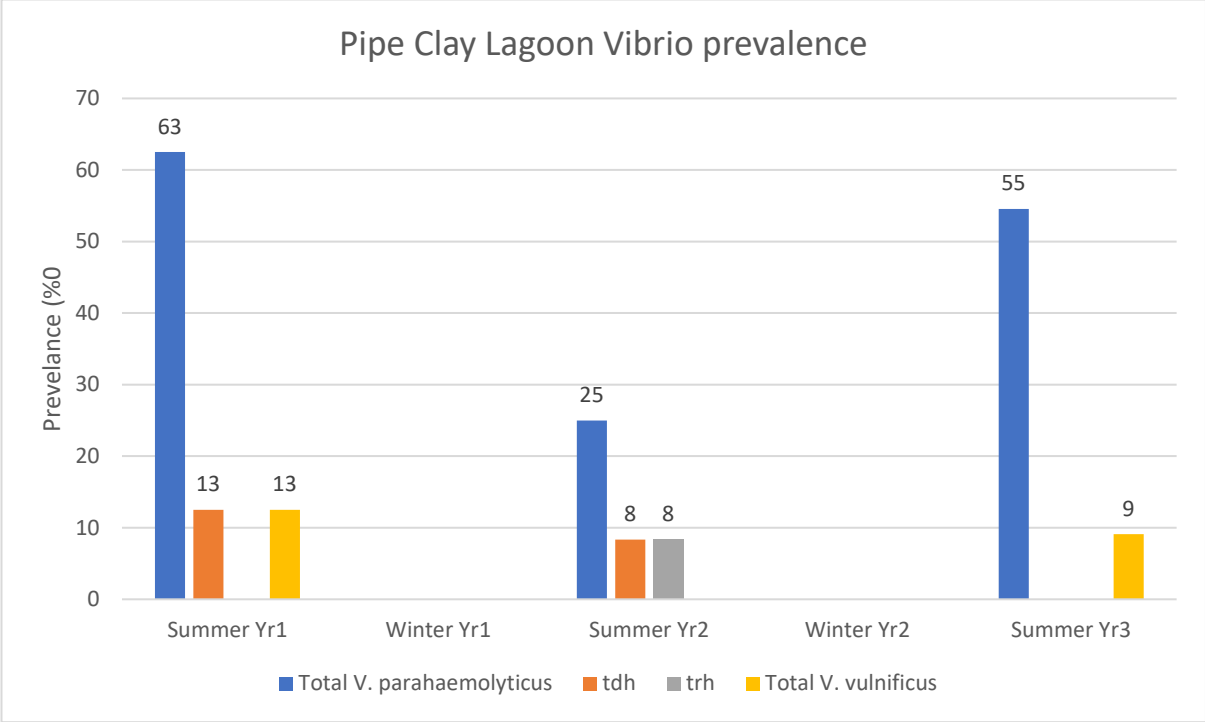

Figure 3: Seasonal *Vibrio* prevalence during the survey period

V. parahaemolyticus was not detected when sampling water temperature was <12°C (Figure 4), the 3-day average water temperature was < 11°C or minimum water temperature 3-days before sampling was <5°C. Most samples collected during the survey were collected below the water (n=27 below, n=12 above and n= 4 not recorded). Of the 14 samples which tested positive for *V. parahaemolyticus* n=9 were collected below the water and n=5 were collected above the water. No relationship could be deduced between tidal stage and *V. parahaemolyticus*; 11 positive samples were collected at high or high outgoing tides and 3 positive samples were collected at low tide (Figure 4).

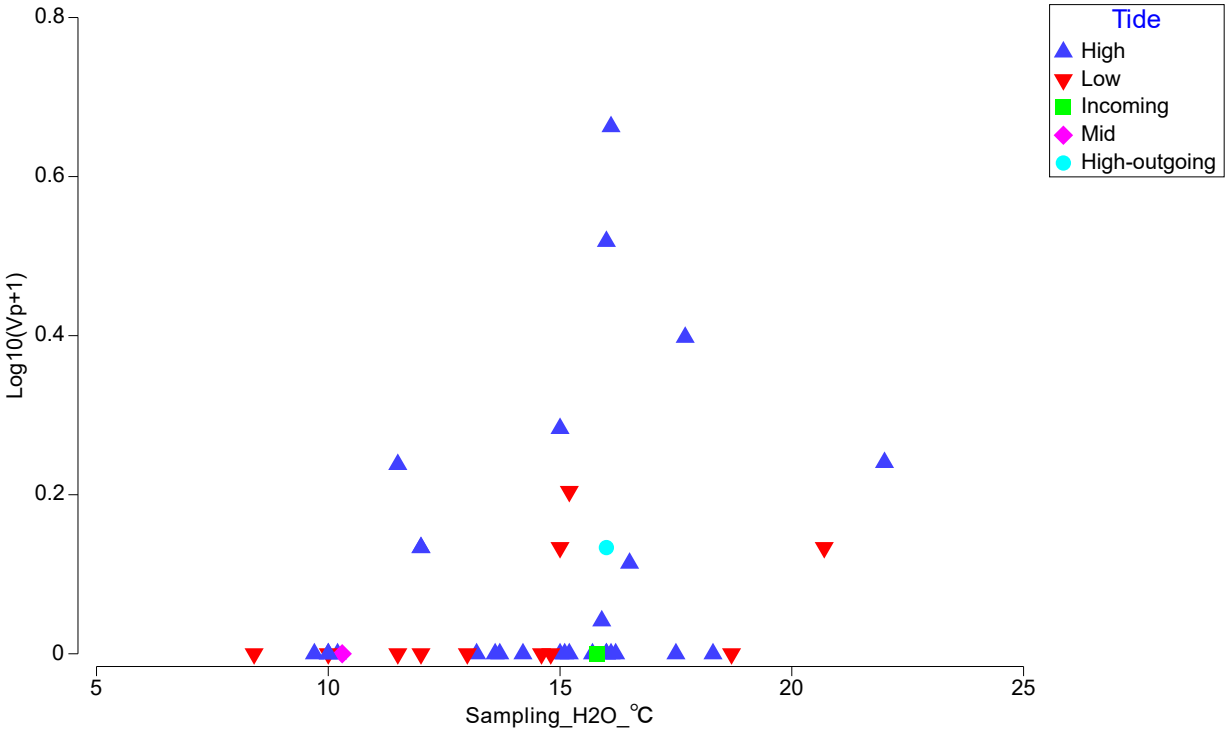

Figure 4: Scatter plot of *V. parahaemolyticus* versus sampling temperature identified by tidal stage of collection.

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data during the survey aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of temperature and rainfall conditions were recorded in the growing area over the period of the survey. Growing water salinity was relatively constant (33-37 PSU). *V. parahaemolyticus* numbers where very low, with a maximum detection of 3.6 MPN/g of oyster. *V. vulnificus* levels were even lower (<1 MPN/g).

Table 1: Summary table of environmental parameters over the summer/autumn and winter/spring survey periods

	Summer (n=31)					**Winter (n=12)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	11.5	22	15.6	2.3	31	8.4	14.6	11.0	1.9	12
Sampling air temp	3.6	19.5	13.0	3.7	16	8.0	13.0	10.8	2.2	6
3-day min H_2O temp	5.0	18.0	11.3	3.3	30	5.0	11.0	7.7	2.3	9
3-day max H_2O temp	16.0	30.0	22.8	3.8	30	10.0	23.0	16.2	4.2	9
3-day Av H_2O temp	11.3	21.1	16.2	2.6	30	9.1	14.5	11.4	1.8	9
1-day max air temp	11.2	35.9	22.1	5.9	31	10.8	21.2	15.0	3.0	12
Salinity (PSU)										
Sampling H_2O	33.7	36.9	35.1	0.8	30	32.7	35.1	34.2	0.7	11
Rainfall (mm)										
1-day	0	11.6	0.8	2.3	31	0	30.2	2.9	8.6	12
2-days	0	22.6	1.6	4.1	31	0	48.0	5.4	13.6	12
3-days	0	49.0	4.8	10.1	31	0	50.2	7.0	14.2	12
7-days	0	61.2	13.4	18.1	31	0	50.6	8.9	14.5	12

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods

***Vibrio* enumeration (MPN/g)**						
Vibrio	**Summer (n=31)**			**Winter (n=12)**		
	Min	**Max**	**Positive (n=)**	**Min**	**Max**	**Positive (n=)**
Total *V. parahaemolyticus*	0	3.6	14	0	0	0
tdh	0	0.36	1	0	0	0
trh	0	0.36	1	0	0	0
Total *V. vulnificus*	0	0.36	2	0	0	0

Statistical analysis

Temperature

All of the temperature predictors were highly positively correlated with one another (Figure 5), particularly the water temperature predictors. Average and minimum water temperature 3 days preceding sampling showed the highest correlation (R=0.89), followed by average and maximum water temperature (R=0.84*). V. parahaemolyticus* numbers showed poor correlation with all the temperature predictors, although a weak positive correlation was noted with sampling water temperature (R=0.28).

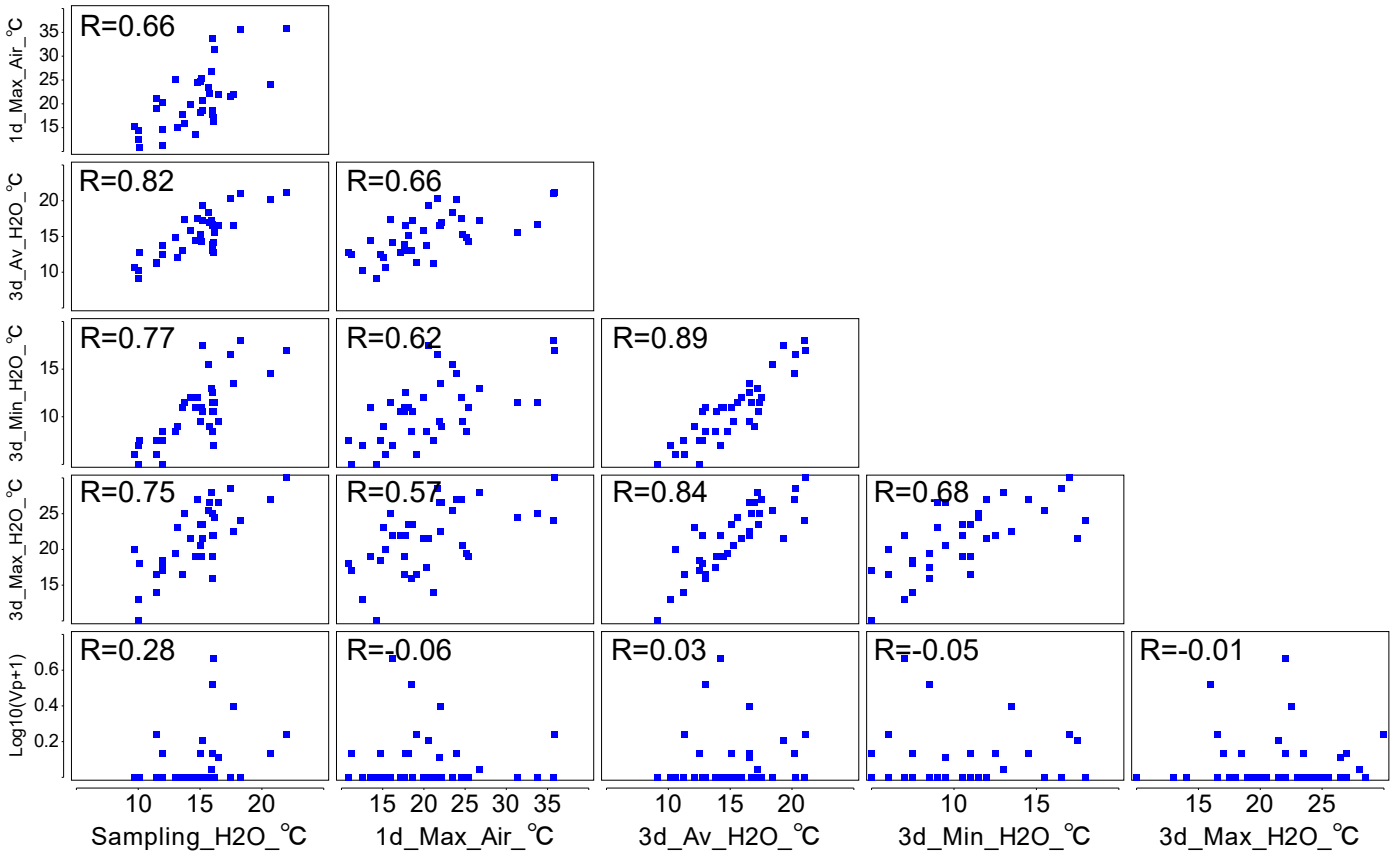

Figure 5: Draftsman's plots of temperature variables and *V. parahaemolyticus*.

Rainfall and salinity

The rainfall predicators were moderately to strongly positively correlated with one another (Figure 7), with the highest correlations observed between 1- and 2-, and 2- and 3-day rainfall (R=0.83). Salinity was somewhat negatively correlated with rainfall, with the highest correlation (R=-0.45) observed between salinity and 3-day rainfall. *V. parahaemolyticus* numbers were poorly correlated with salinity or any of the rainfall predictor variable, although some positive correlation was observed with 7-day rainfall (R=0.30).

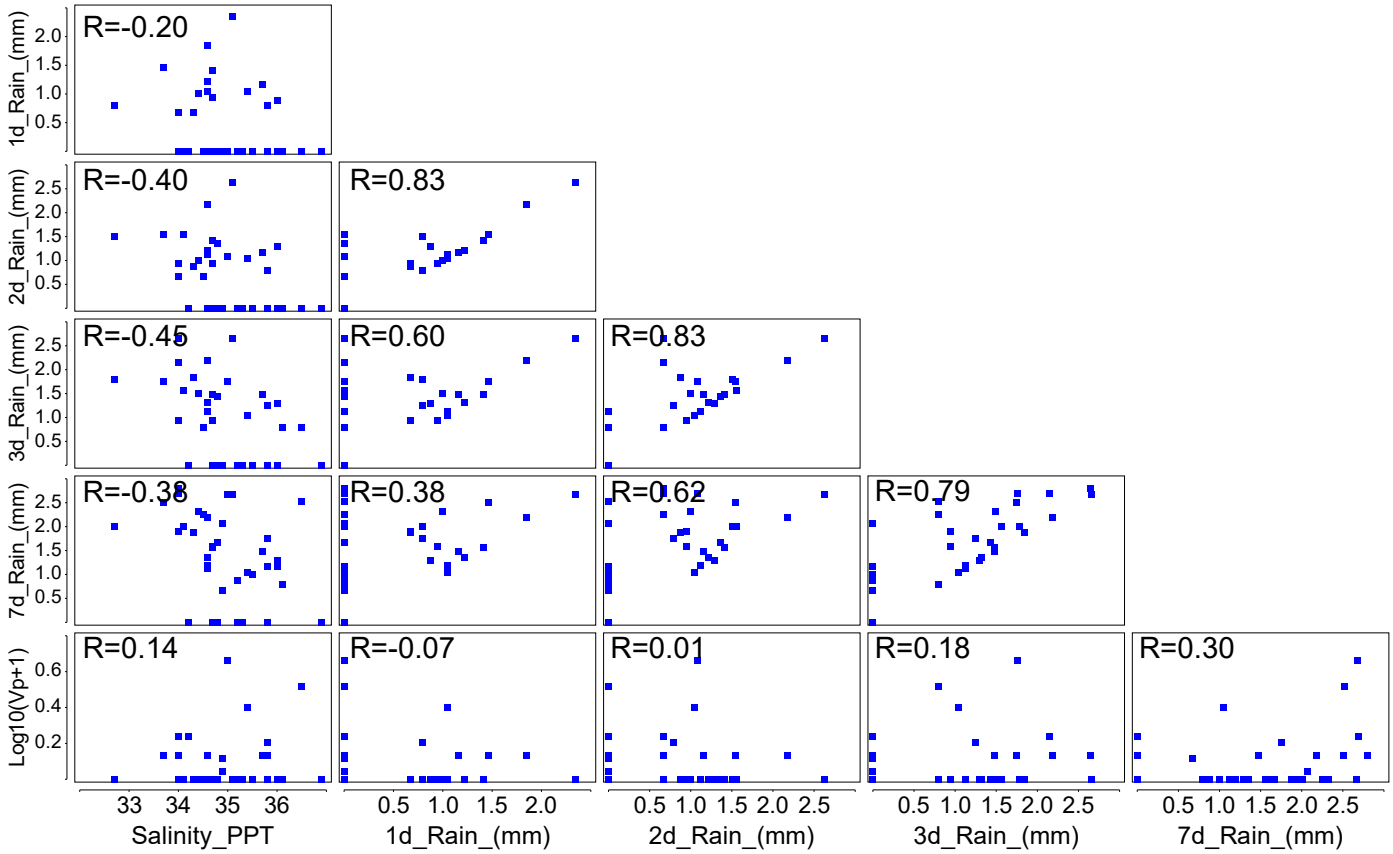

Figure 6: Draftsman's plots of rainfall and salinity variables and *V. parahaemolyticus*.

Linear predictive model generation

The marginal statistical tests in DISTLM showed no significant (P<0.05) relationships with any of the predictor variables individually and *V. parahaemolyticus* numbers. Although sampling water temperature (Pseudo-F=3.1, P=0.092) and 7-day rainfall (Pseudo-F=3.5, P=0.070) were trending towards significance with *V. parahaemolyticus* numbers, accounting for 8% and 9% of the explained variability in the data set. In the most accurate predictive model selection sampling water temperature and 7-day rainfall preceding sampling described 100% of the explained variability in the fitted model, 29% of the total variability in the data cloud (AICc=-144.94 R^2=0.29). In this model, once 7-day rainfall had been fitted in the model, sampling water temperature explained an additional 20% of the variability (Figure 7). It should be noted that hypothesis testing (e.g., marginal statistical tests) and model selection (e.g., with AICc) serve different purposes. P-values measure evidence against a null hypothesis, usually of "no effect". This is a conservative approach, putting the burden of evidence on the data to demonstrate an effect. AICc aims to select the model that will make the most accurate predictions for new data. Having said that the coefficient of determination (R^2) is low indicating that the developed model is not very robust for Pipe Clay Lagoon.

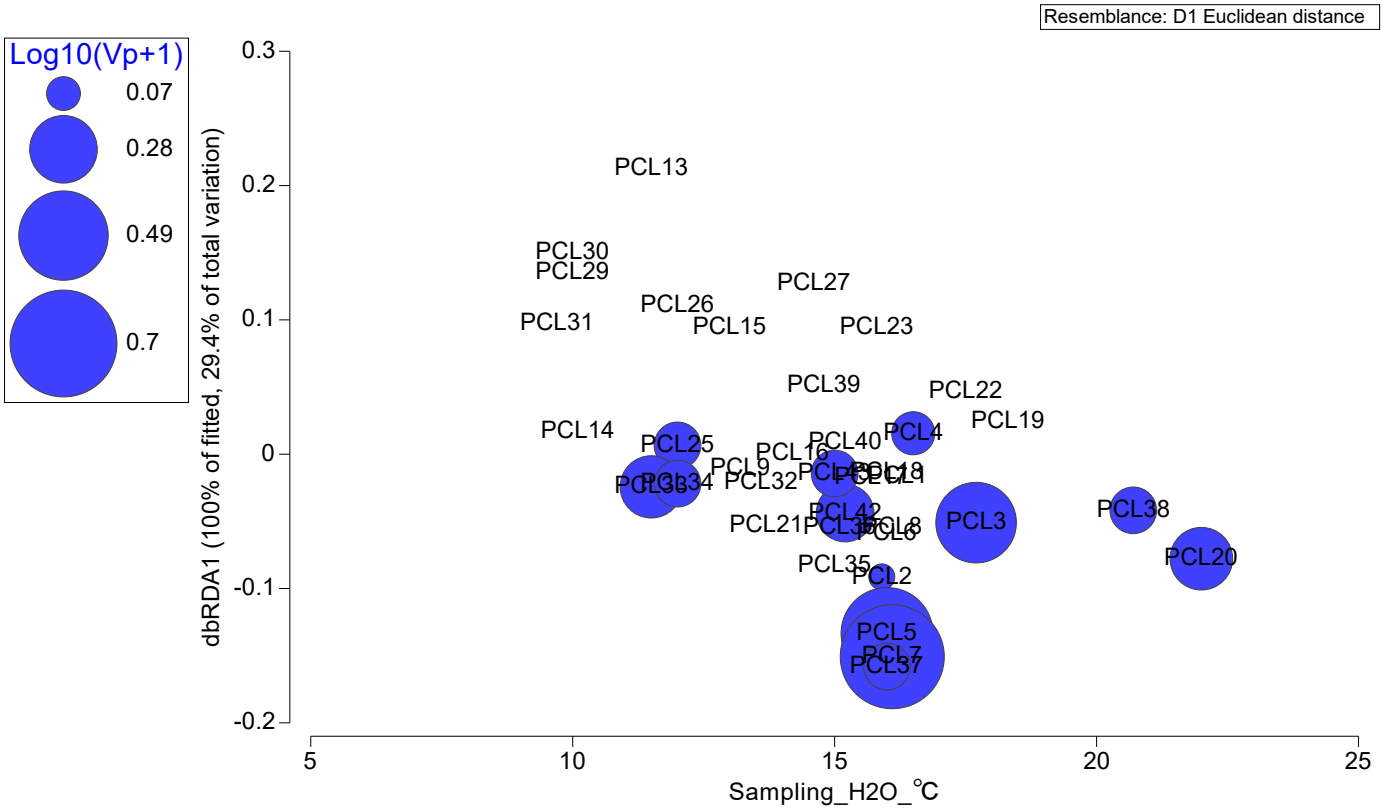

Figure 7: Distance based redundancy analysis (dbRDA) to visualise the DISTLAM results of the developed model with the predictor variables of sampling water temperature and 7-day rainfall prior to sampling. Bubbles on plot indicate level of *V. parahaemolyticus* (Vp) detected in shellfish sampled from Pipe Clay Lagoon.

The predictive model developed for *V. parahaemolyticus* in Pipe Clay Lagoon for oysters was based on 7-day rainfall prior to sampling and sampling water temperature:

$$y = -0.4480 + 0.0881\sqrt[4]{x_0} + 0.0277x_1$$

Where:

y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = 7-day rainfall prior to sampling (mm)
x_1 = sampling water temperature (°C)
R^2 = 0.29

Conclusions

- *V. parahaemolyticus* was only detected in Pipe Clay Lagoon oysters during the summer/autumn sampling periods, with a prevalence of 25-63%.
- Levels of *V. parahaemolyticus* detected during the survey period were low; ≤3.6 MPN/g of oysters.
- *V. parahaemolyticus* carrying the *tdh* and *trh* genes, which are often associated with clinical strains, were only detected in the first two summer/autumn seasons with a prevalence of 8-13% for *tdh* and 0-8% for *trh*.
- *V. vulnificus* was only detected during the first and third summer/autumn sampling periods, and levels were <1 MPN/g of oysters. In these two positive samples salinity of sampling water temperature were 35 PSU and 16°C, and 34 PSU and 12°C.
- Sampling water temperature and 7-day rainfall prior to oyster harvest were trending towards being significant predictors for *V. parahaemolyticus* numbers individually, although not significant. The best predictive model developed for *V. parahaemolyticus* risk in Pipe Clay Lagoon incorporated both 7-day rainfall and sampling water temperature. It should be noted that the coefficient of determination (R^2) is low indicating that the developed model is not very robust for Pipe Clay Lagoon.

$$y = -0.4480 + 0.0881\sqrt[4]{x_0} + 0.0277x_1$$

Where:
y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = 7-day rainfall prior to sampling (mm)
x_1 = sampling water temperature (°C)
R^2 = 0.29

Appendix 11: *Vibrio* Survey Great Bay, 2020-2022

Background

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Great Bay (Figure 1).

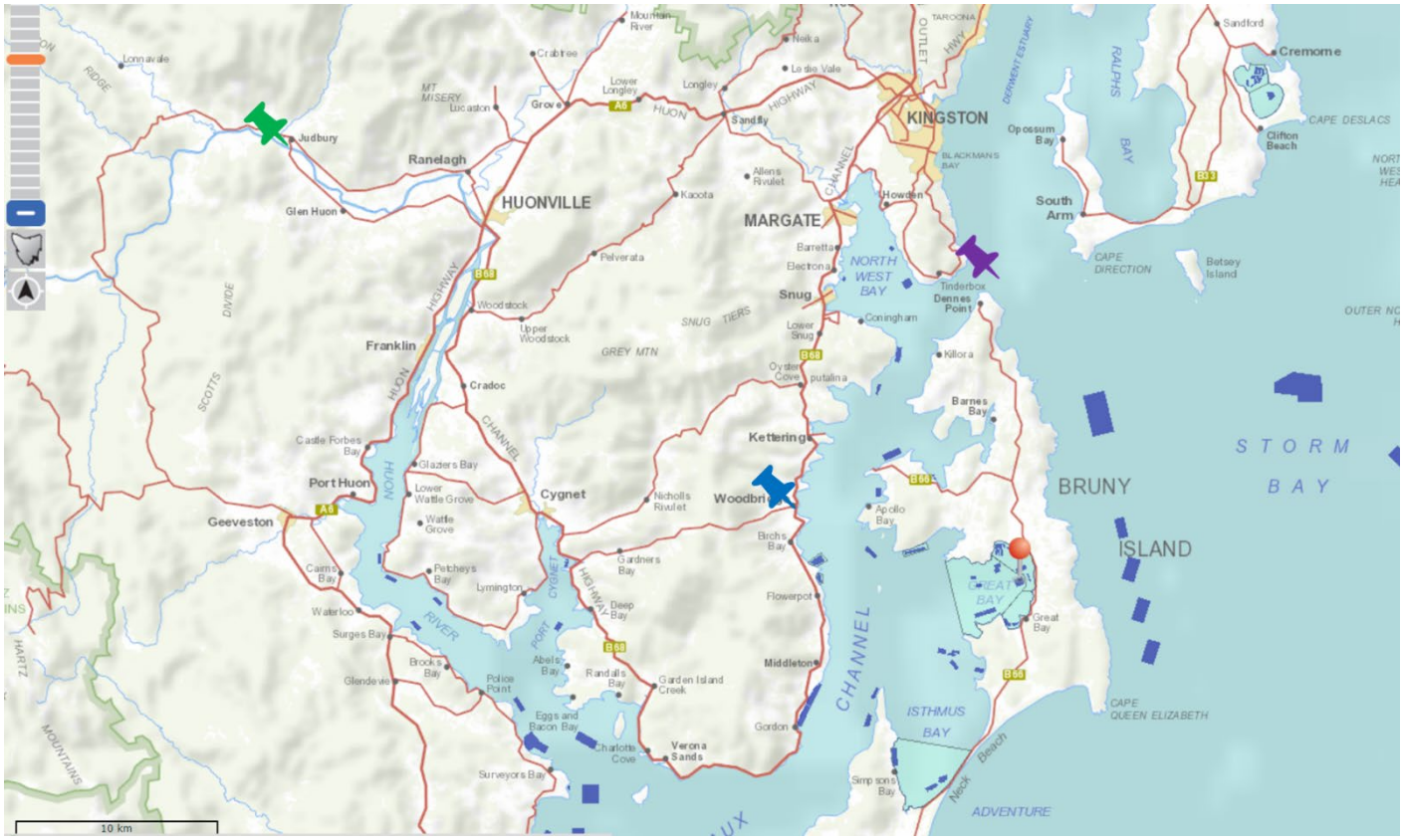

Figure 1: Great Bay harvest area. Pin indicates lease from which sampling occurred. Green pin indicates Huon River flow station at D/S Judbury Bridge. Blue pin indicates BOM rainfall station at Woodbridge. Purple pin indicates BOM temperature station at Dennes Point. Scale bar = 10 km.

Methodology

Summary of survey design

Oysters were sampled over three summer/autumn (November to April) and two winter/spring (May to October) periods from January 2020 to end of April 2022. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data collected: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data collected: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity, water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation. Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station number 94255; Dennes Point). Maximum river flow on the day of sampling was also accessed from the Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable, for the river identified in the harvest areas' sanitary survey. For Great Bay the river input identified was the Huon

River. Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 94068; Woodbridge).

Statistical analysis

Statistical analysis of biological and environmental data was done in Primer 7 + PERMANOVA (Primer-e). Correlation among variables was investigated using draftsman's plots. *V. parahaemolyticus* (MPN/g) data were $\log_{10}(X+1)$ transformed and used to create a Euclidean distance resemblance matrix. The environmental data was also transformed if not normally distributed: rainfall data was square root transformed while river flow data was fourth root transformed. The DISTLM (distance-based linear model) routine was used to investigate the relationship between the *V. parahaemolyticus* Euclidean distance resemblance matrix and the environmental (temperature, salinity, rainfall and river flow) predictor variables. The step-wise "selection procedure" was used with the Akaike Information Criterion corrected for small sample sizes (AICc) "selection criterion" and 9999 permutations. AICc is a mathematical method for evaluating how well a model fits the data it was generated from. The output of this analysis gives a statistical marginal test for each individual predictor variable considered alone, and the best overall solution. The best linear model was visually represented using dbRDA (distance-based redundancy analysis). The formula for the resulting predictive model was generated using the regression function in excel.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Salinity and rainfall data during the survey period are shown in Figure 2A. Significant rainfall events were observed in all seasons. Increased river flow of the Huon River was observed during the winter/spring (Figure 2B). Figure 2C shows *V. parahaemolyticus* levels, sampling water temperature and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Temperature and *V. parahaemolyticus* numbers increased during summer/autumns months. Salinity in Great Bay was relatively constant (31-36 PSU). An unexplained outlying salinity was recorded for sample GB13 (44 PSU) which was removed from subsequent analysis (Figure 2A).

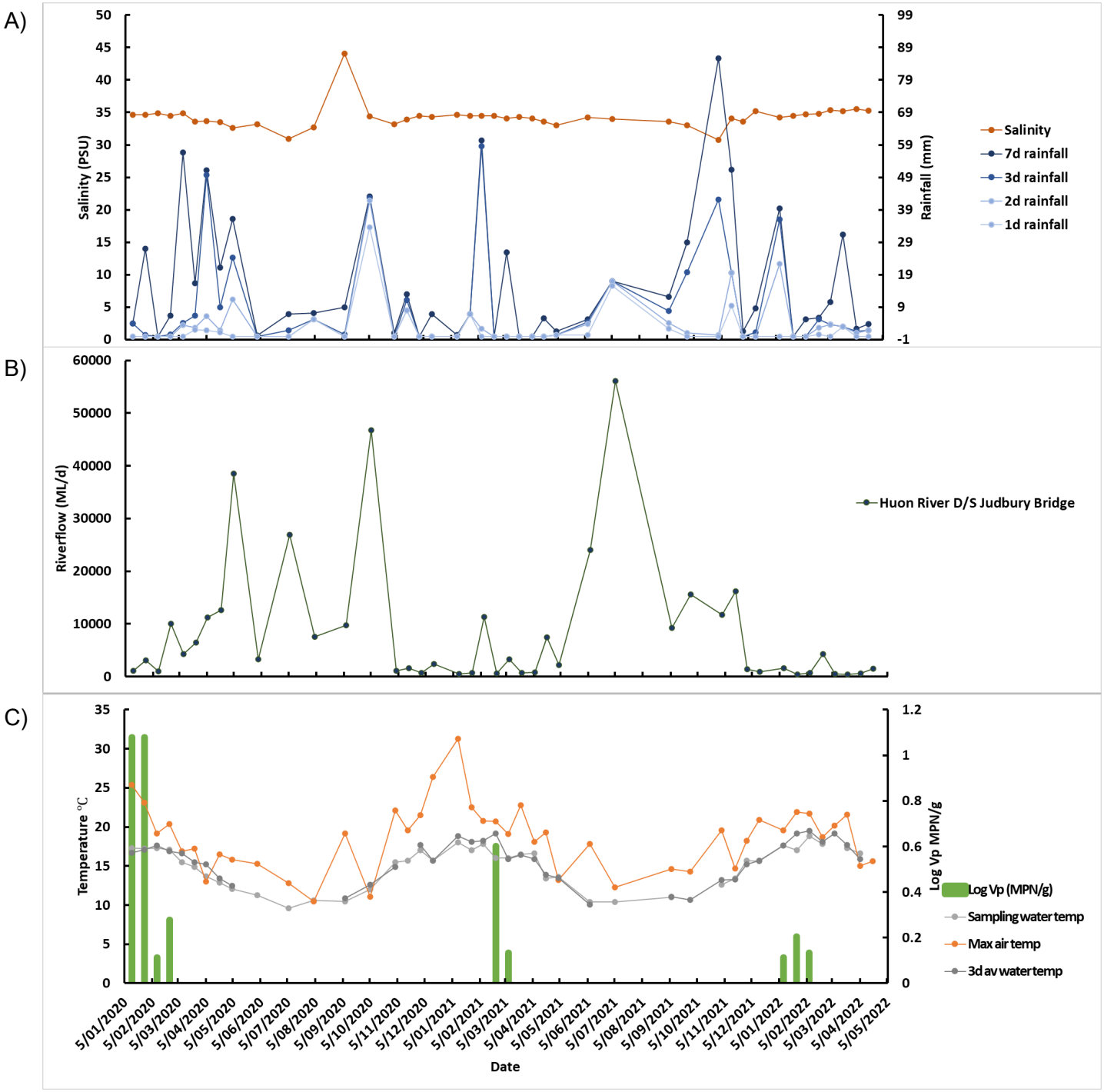

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Great Bay. 2A shows water salinity at sampling and rainfall at Woodbridge (1, 2, 3 and 7 days) prior to sampling. 2B shows maximum river flow 24 hours prior to sampling. 2C shows levels of *V. parahaemolyticus* detected, overlayed with sampling water temperature, three-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence for Great Bay broken down to the 5 seasons sampled during the survey. A low to moderated prevalence of *V. parahaemolyticus* was detected during the summer/autumn seasons (8-50%), No *V. parahaemolyticus* was detected in the winter/spring. *V. parahaemolyticus* strains containing the pathogenicity associated *tdh* gene was only detected in the first summer/autumn sampling period with a prevalence of 13%. *No V. vulnificus* was detected during the survey period.

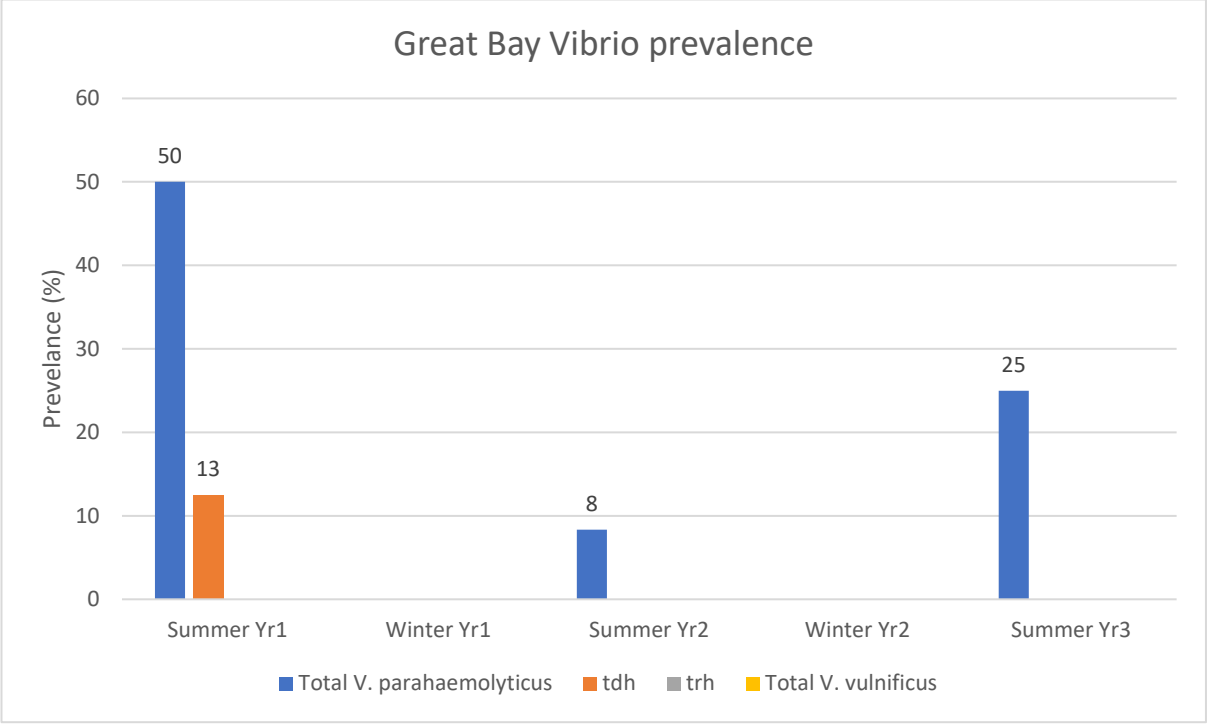

Figure 3: Seasonal *Vibrio* prevalence during the survey period.

No *V. parahaemolyticus* positive samples were detected when the sampling water temperature was <16°C (Figure 4), average water temperature and minimum water temperature 3-days before sampling were <15°C and <13°C, respectively. Of the 40 samples analysed 39 were collected below the water and one sample had no recorded information on the sample submission form. No clear relationship between tidal stage and *V. parahaemolyticus* levels could be deducted (Figure 4). Of the 8 samples which tested positive during the survey period, n=5 were collected at high-, n=2 at low- tide and n=1 at mid-tide (Figure 4).

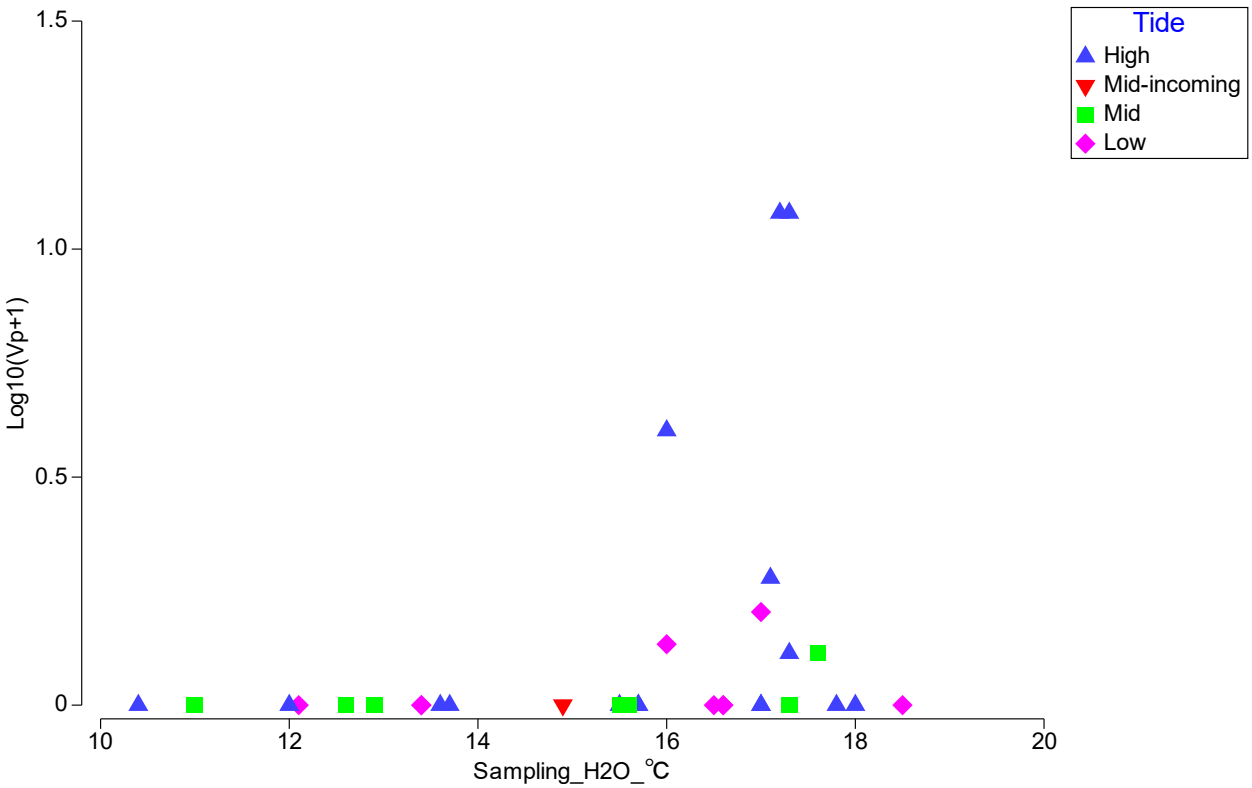

Figure 4: Scatter plot of *V. parahaemolyticus* versus sampling temperature identified by tidal stage of collection.

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data during the survey aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of environmental conditions were recorded in the growing area over the period of the survey. Low levels of up to 11 MPN/g *V. parahaemolyticus* were detected during the survey.

Table 1: Summary table of environmental parameters over the summer/autumn and winter/spring survey periods

	Summer (n=29)					**Winter (n=11)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	12.6	18.5	16.1	1.6	28	9.6	13.6	11.1	1.1	11
Sampling air temp	12.0	21.0	15.8	3.3	12	7.0	12.0	10.5	2.4	4
3-day min H_2O temp	10.5	18.5	15.0	2.1	28	8.5	13.0	11.0	1.6	7
3-day max H_2O temp	14.0	29.0	18.3	3.2	28	10.5	13.5	12.3	1.1	7
3-day Av H_2O temp	13.2	19.2	16.5	1.8	28	10.1	13.5	11.6	1.2	7
1-day max air temp	13.0	31.3	20.1	3.8	29	10.5	19.2	14.2	2.7	11
Salinity (PSU)										
Sampling H_2O	30.8	35.5	34.3	0.9	29	30.9	34.4	33.1	1.0	10
Rainfall (mm)										
1-day	0	9.4	0.8	2.2	29	0	33.6	5.2	10.5	11
2-days	0	22.4	2.6	5.4	29	0	41.8	7.7	12.5	11
3-days	0	58.6	8.6	16.3	29	0	42.6	11.3	13.4	11
7-days	0	85.6	18.1	23.1	29	0.2	43.2	15.2	14.5	11
River flow (ML/d)										
Huon River	406	16226	3940.8	4696.1	29	2157.2	56088	21798	18396	11

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods

***Vibrio* enumeration (MPN/g)**						
Vibrio	**Summer (n=29)**			**Winter (n=11)**		
	Min	**Max**	**Positive (n=)**	**Min**	**Max**	**Positive (n=)**
Total *V. parahaemolyticus*	0	11	8	0	0	0
tdh	0	11	1	0	0	0
trh	0	0	0	0	0	0
Total *V. vulnificus*	0	0	0	0	0	0

Statistical analysis

Temperature

All the temperature predictor variables were strongly positively correlated with one another, with the water temperature predictor variables showing the highest inter-correlations (Figure 5). The 3-day average water temperature prior to sampling was most correlated with sampling water temperature (R=0.95), followed by the minimum water temperature 3-days prior to sampling (R=0.90). *V. parahaemolyticus* was positively correlated with temperature and showed the strongest correlation with daily air temperature (R=0.35).

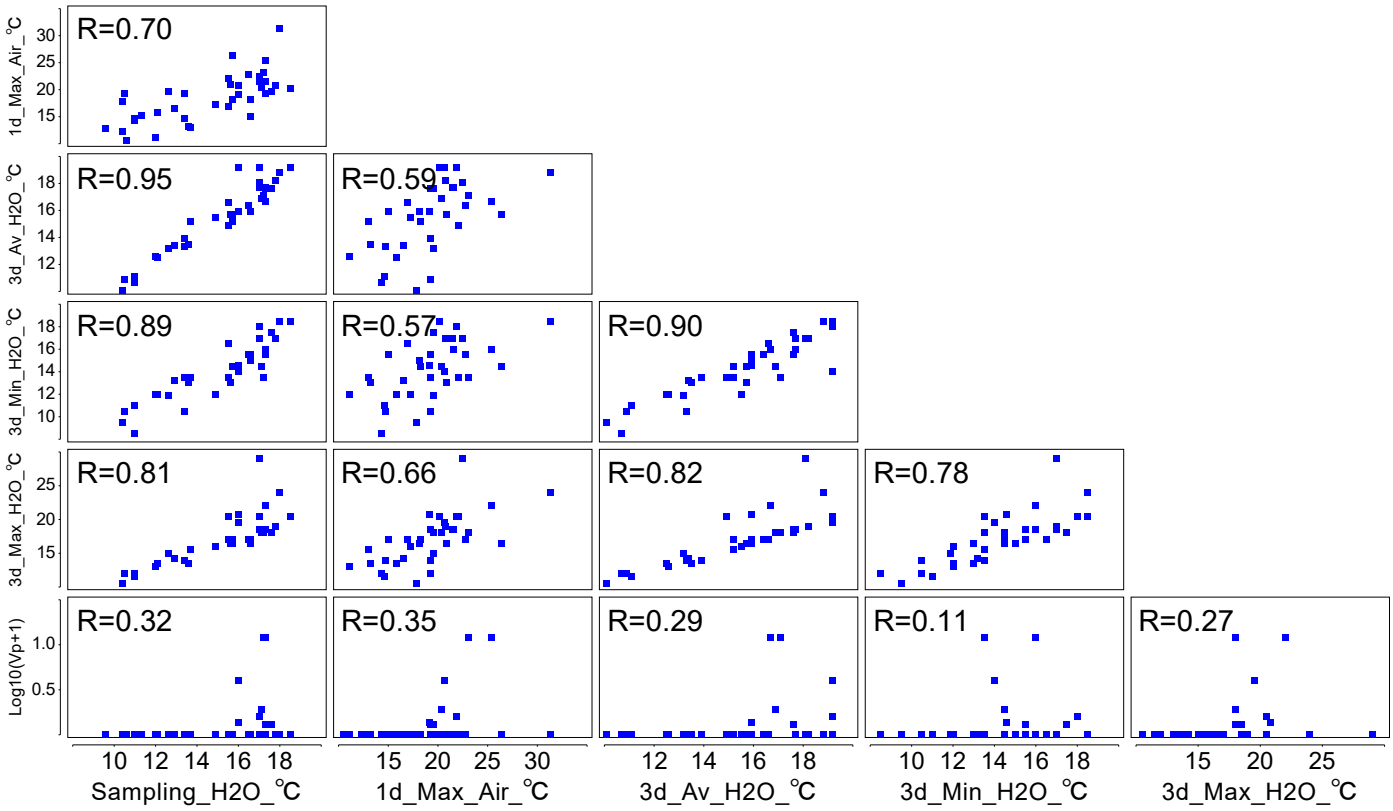

Figure 5: Draftsman's plots of temperature variables and *V. parahaemolyticus*.

Rainfall

All the rainfall predictor variable were moderately to strongly correlated with one another (Figure 6). The highest inter-correlation was observed between 3- and 7-day cumulative rainfall (R=0.82).

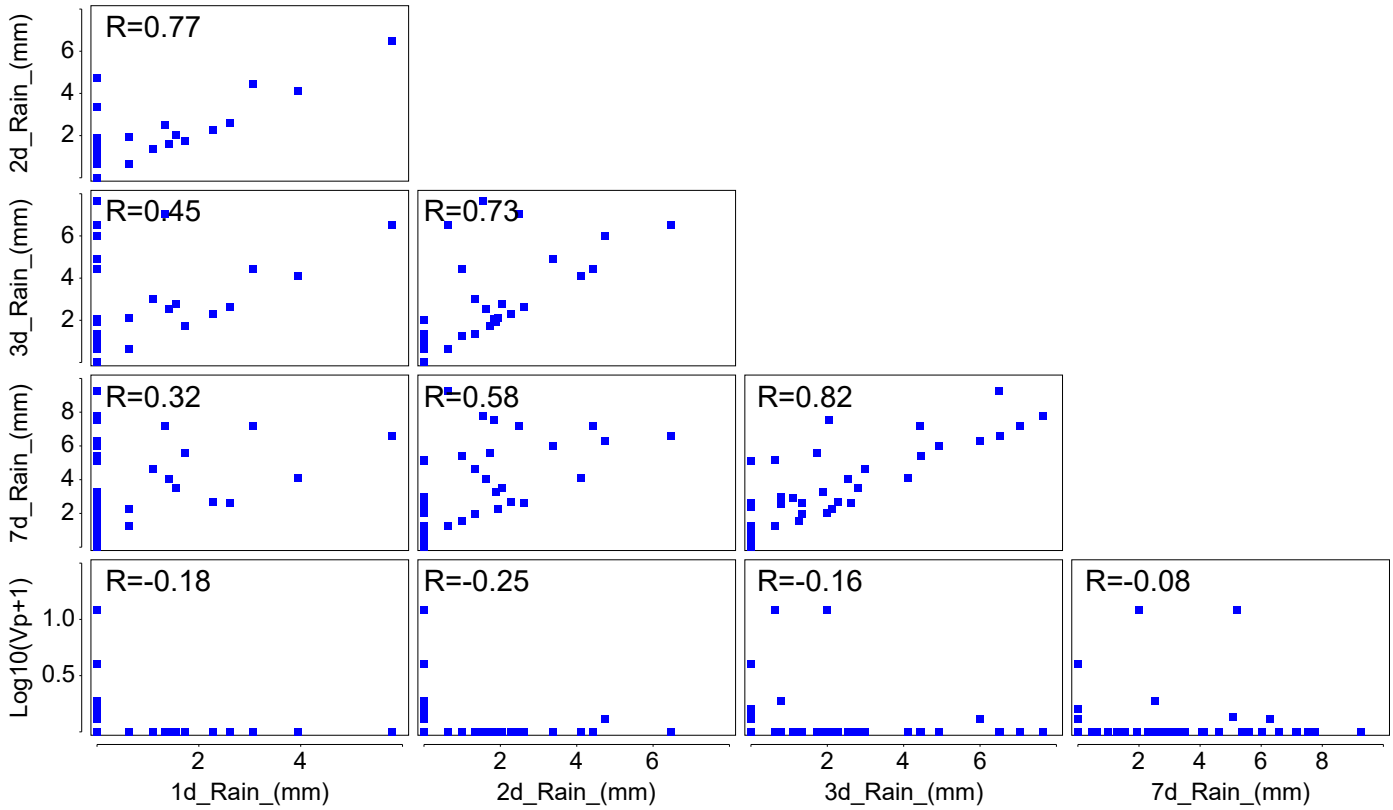

Figure 6: Draftsman's plots of rainfall variables and *V. parahaemolyticus*. Rainfall data was square root transformed.

Salinity and river flow

Great Bay harvest water salinity and the Huon River flow were negatively correlated (Figure 7). *V. parahaemolyticus* numbers were weakly positively correlated harvest water salinity and weakly negatively correlated with river flow.

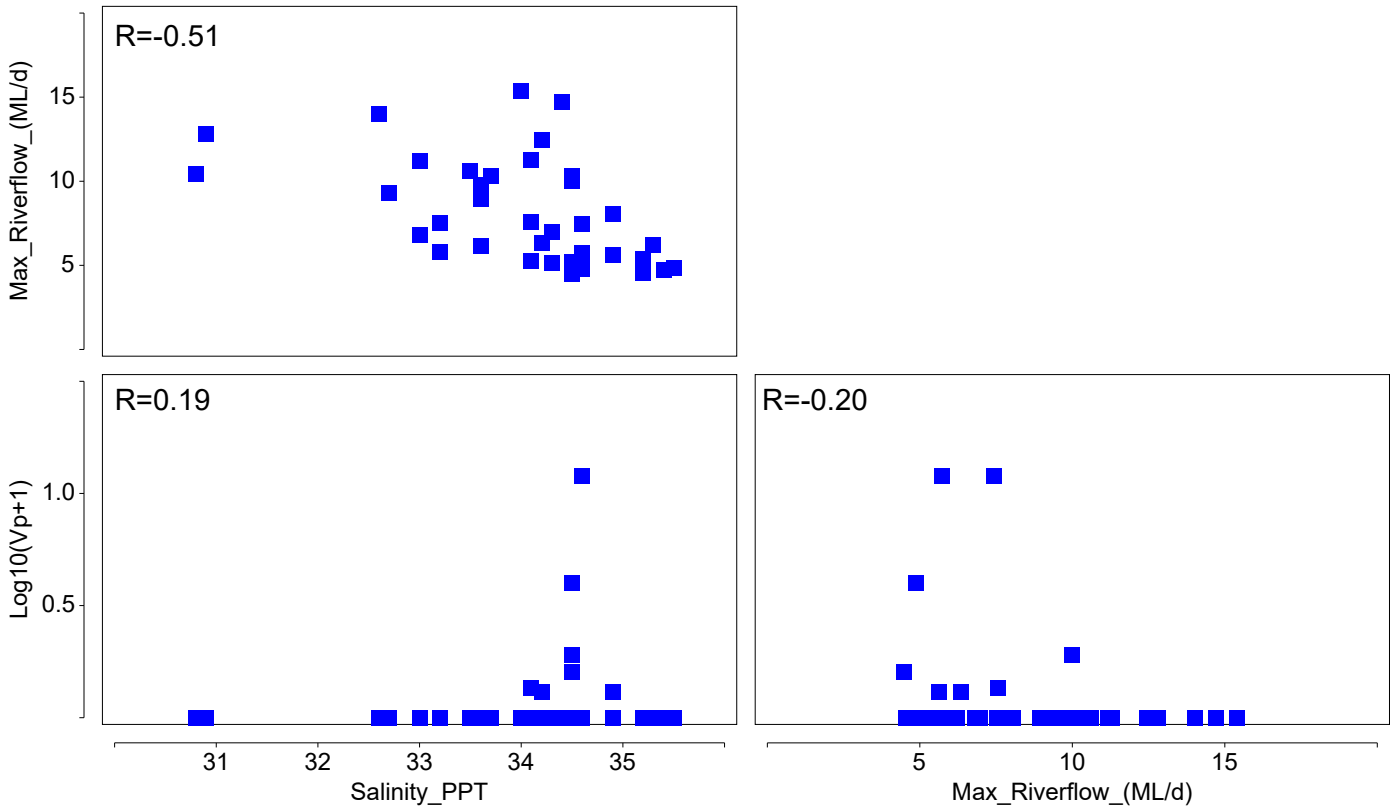

Figure 7: Draftsman's plots of salinity, river flow and *V. parahaemolyticus* variables. River flow data was fourth root transformed.

Linear predictive model generation

The marginal statistical tests in DISTLM showed no significant (P<0.05) relationships with any of the predictor variables and *V. parahaemolyticus* numbers individually. Although sampling water temperature (Pseudo-F=3.1, P=0.076), daily maximum air temperature (Pseudo-F=4.0, P=0.059) and 3-day average water temperature prior to sampling (Pseudo-F=2.9, P=0.092) were trending towards significance with *V. parahaemolyticus* numbers, accounting for 9%, 11 and 8% of the explained variability in the data set. In the most accurate predictive model selection maximum daily air temperature described 100% of the explained variability in the fitted model, although only predicting 11% of the total variability in the data cloud (AICc=-88.742 R^2=0.11) (Figure 8).

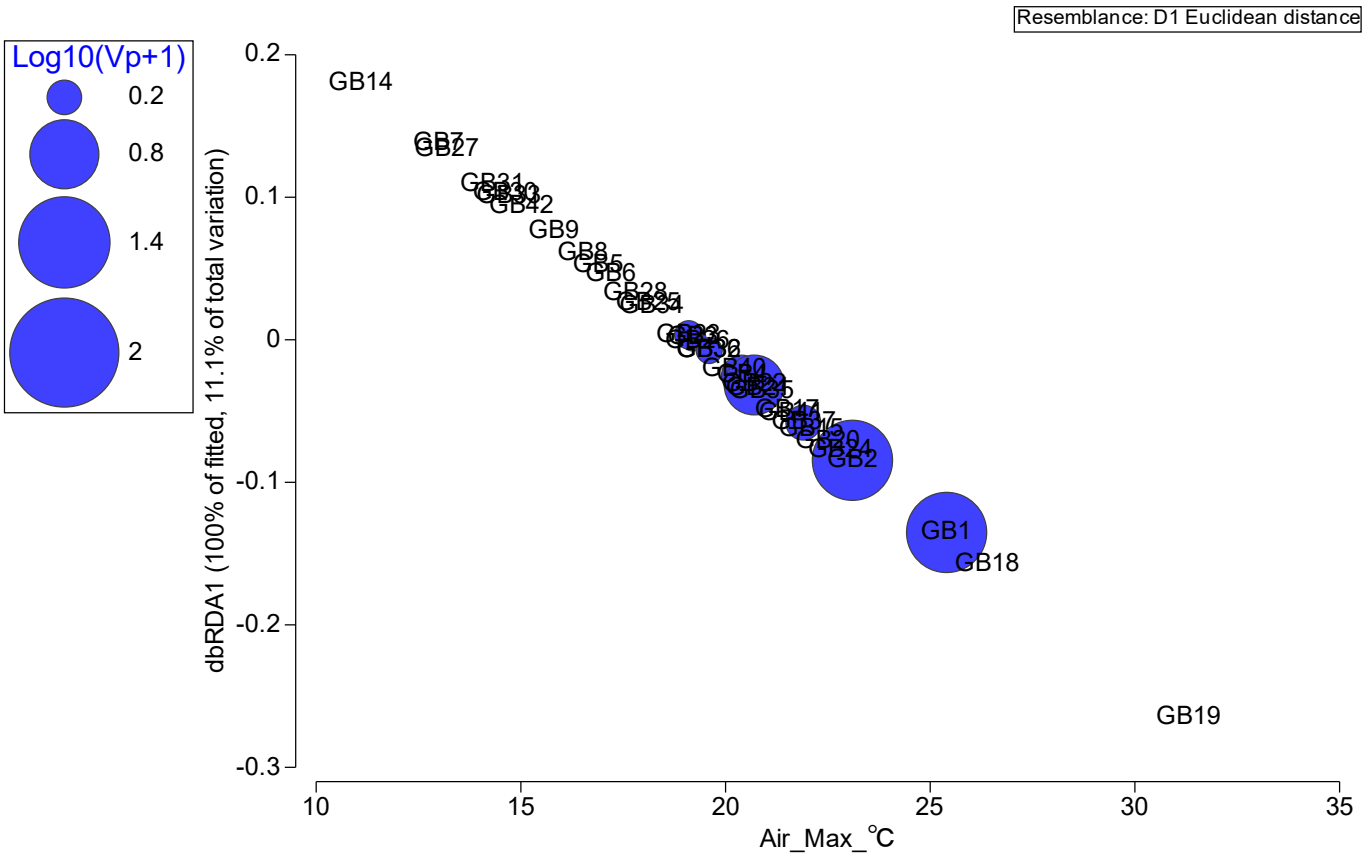

Figure 8: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with the predictor variable of maximum daily air temperature. Bubbles on plot indicate level of *V. parahaemolyticus* (Vp) detected in oysters from Great Bay.

The predictive model developed for *V. parahaemolyticus* in Great Bay for oysters was based on the maximum daily air temperature:

$$y = -0.2891 + 0.0205x_0$$

Where:

y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = maximum daily air temperature (°C)
R^2 = 0.11

Conclusions

- *V. parahaemolyticus* was only detected in Great Bay oysters during the summer/autumn sampling periods, with a prevalence of 8-50%. No *V. parahaemolyticus* positive samples were detected when the sampling water temperature was <16°C, average water temperature and minimum water temperature 3-days before sampling were <15°C and <13°C, respectively. Although, these temperature predictors were not significantly associated with *V. parahaemolyticus* in Great Bay.
- Levels of *V. parahaemolyticus* detected during the survey period were low; ≤11 MPN/g of oysters.
- *V. parahaemolyticus* strains carrying the *tdh* gene were only detected in the first summer/autumn season with a prevalence of 13%.
- *V. vulnificus* was not detected in Great Bay during the survey.
- Sampling water temperature, 3-day average water temperature prior to sampling and maximum daily air temperature were trending towards being significant predictors for *V. parahaemolyticus* numbers individually. The best predictive model developed for *V. parahaemolyticus* risk in Great Bay was maximum daily air temperature, although this variable only predicted 11% of the variability observed.

$$y = -0.2891 + 0.0205x_0$$

Where:
y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = maximum daily air temperature (°C)
R^2 = 0.11

Appendix 12: *Vibrio* Survey Fleurtys Point, 2020-2022

Background

Harvest area

During the *Vibrio* survey, Pacific Oysters were sampled from a single lease in Fleurtys Point (Figure 1).

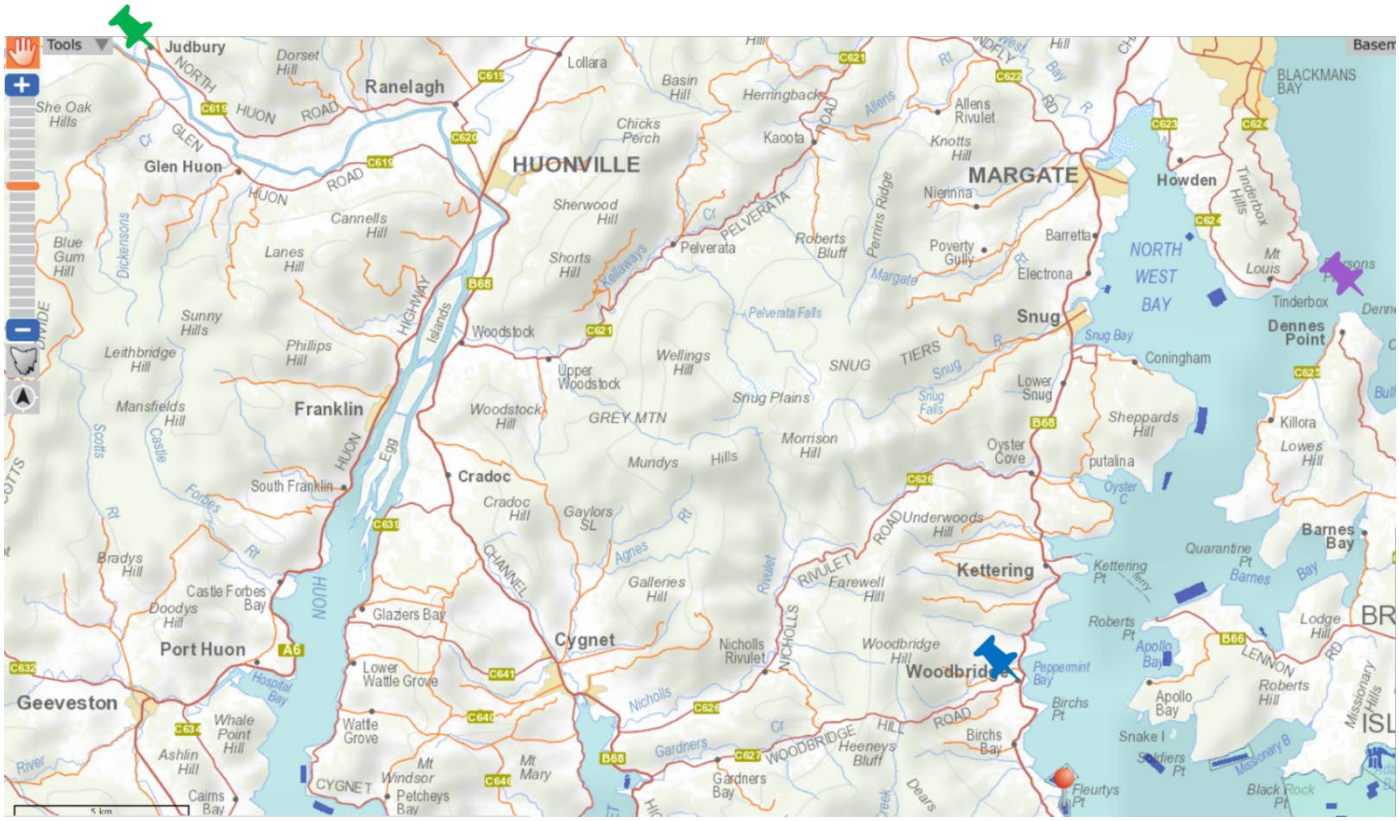

Figure 1: Fleurtys Point harvest area. Orange pin indicates lease from which sampling occurred. Green pin indicates Huon River flow station at D/S Judbury Bridge. Purple pin indicates BOM temperature station at Dennes Point. Blue pin indicates BOM rainfall station at Woodbridge. Scale bar = 5 km.

Methodology

Summary of survey design

Oysters were sampled over two summer/autumn (November to April) and one winter/spring (May to October) periods from December 2020 to end of April 2022. During the summer/autumn period oysters were sampled fortnightly, while during the winter/spring period shellfish were sampled monthly.

Biological data collected: Enumeration (MPN/g) of total *Vibrio parahaemolyticus* and potential pathogenicity determinants *tdh*/*trh*. Enumeration of total *Vibrio vulnificus*.

Environmental data collected: Temperature data loggers were used to log growing water temperature prior to sampling and during transit of samples to the Public Health Laboratory in Hobart. Temperature data from the loggers were used to calculate a 3-day average water temperature preceding sampling and maximum and minimum water temperature during that period. At sampling, salinity, water and air temperature was recorded by the sampler on the sample submission form. A water sample was also provided for salinity confirmation. Maximum air temperature on the day of collection was obtained from the Bureau of Meteorology (BOM) (Station number 94255; Dennes Point). Maximum river flow on the day of sampling was also accessed from the Department of Natural Resources and Environment (NRE) Tasmanian Water Information web portal, if applicable, for the river identified in the harvest areas' sanitary survey. For Fleurtys Point harvest area this was the Huon

River. Rainfall 1, 2, 3 and 7 days prior to shellfish collection was obtained from the BOM (Station number 94068; Woodbridge).

Statistical analysis

Statistical analysis of biological and environmental data was done in Primer 7 + PERMANOVA (Primer-e). Correlation among variables was investigated using draftsman's plots. *V. parahaemolyticus* (MPN/g) data were $\log_{10}(X+1)$ transformed and used to create a Euclidean distance resemblance matrix. The environmental data was also transformed if not normally distributed: rainfall data for Fluertys Point was square root transformed while river flow data was fourth root transformed. The DISTLM (distance-based linear model) routine was used to investigate the relationship between the *V. parahaemolyticus* Euclidean distance resemblance matrix and the environmental (temperature, salinity and rainfall) predictor variables. The step-wise "selection procedure" was used with the Akaike Information Criterion corrected for small sample sizes (AICc) "selection criterion" and 9999 permutations. AICc is a mathematical method for evaluating how well a model fits the data it was generated from. The output of this analysis gives a statistical marginal test for each individual predictor variable considered alone, and the best overall solution. The best linear model was visually represented using dbRDA (distance-based redundancy analysis). The formula for the resulting predictive model was generated using the regression function in excel.

Results

Local environmental drivers of *V. parahaemolyticus* levels

Fleurtys Point was only sampled over two summer/autumn and one winter/spring periods. Salinity and rainfall data during the survey period are shown in Figure 2A. The highest rainfall events were recorded in spring and summer months. Increased flow of the Huon River by contrast was observed in the winter (Figure 2B). Figure 2C shows *V. parahaemolyticus* levels, sampling water temperature and maximum air temperature on the day of sampling, along with the 3-day average water temperature preceding sampling. Temperature and *V. parahaemolyticus* numbers increased during summer/autumns months. Salinity at Fleurtys Point was relatively constant (30-35 PSU).

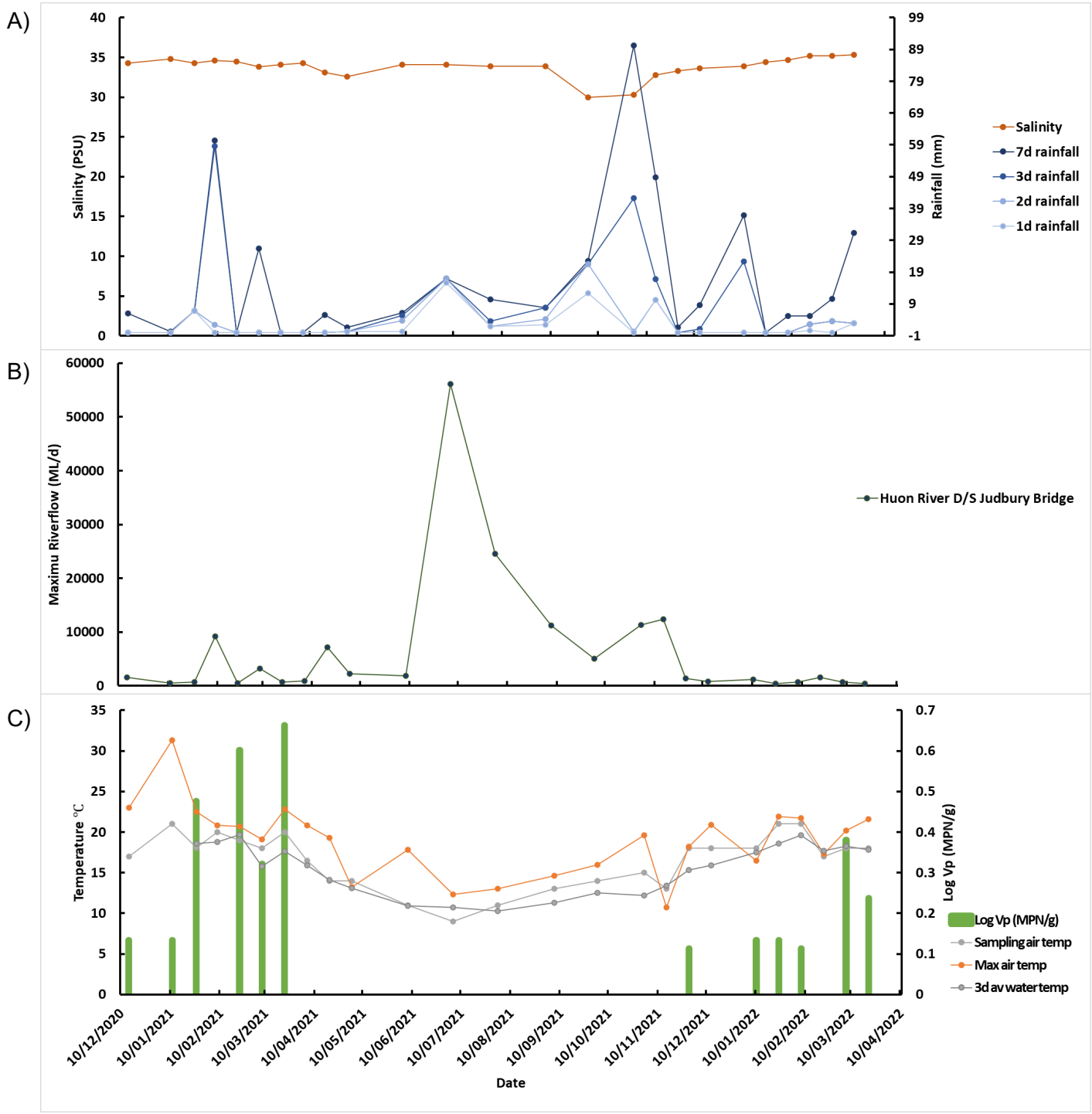

Figure 2: Environmental and *V. parahaemolyticus* (Vp) survey results for Fleurtys Point. 2A shows water salinity at sampling and rainfall (1, 2, 3 and 7 days) prior to sampling. 2B shows maximum river flow 24 hours prior to sampling. 2C shows levels of *V. parahaemolyticus* detected and are overlayed with sampling water temperature, three-day average water temperature prior to sampling and maximum air temperature on day of sampling.

Prevalence of total *V. parahaemolyticus*, *tdh* and *trh*, and total *V. vulnificus* over survey

Figure 3 shows the *Vibrio* prevalence for Fleurtys Point broken down to the 3 seasons sampled during the survey. *V. parahaemolyticus* was only detected during the summer/autumn sampling periods with a prevalence ranging from 60-67%. *V. parahaemolyticus* strains containing the pathogenicity associated genes *tdh* and *trh* were only detected in the last summer/autumn sampling period with a prevalence of 20% and 10% respectively. No *V. vulnificus* was detected during the survey period.

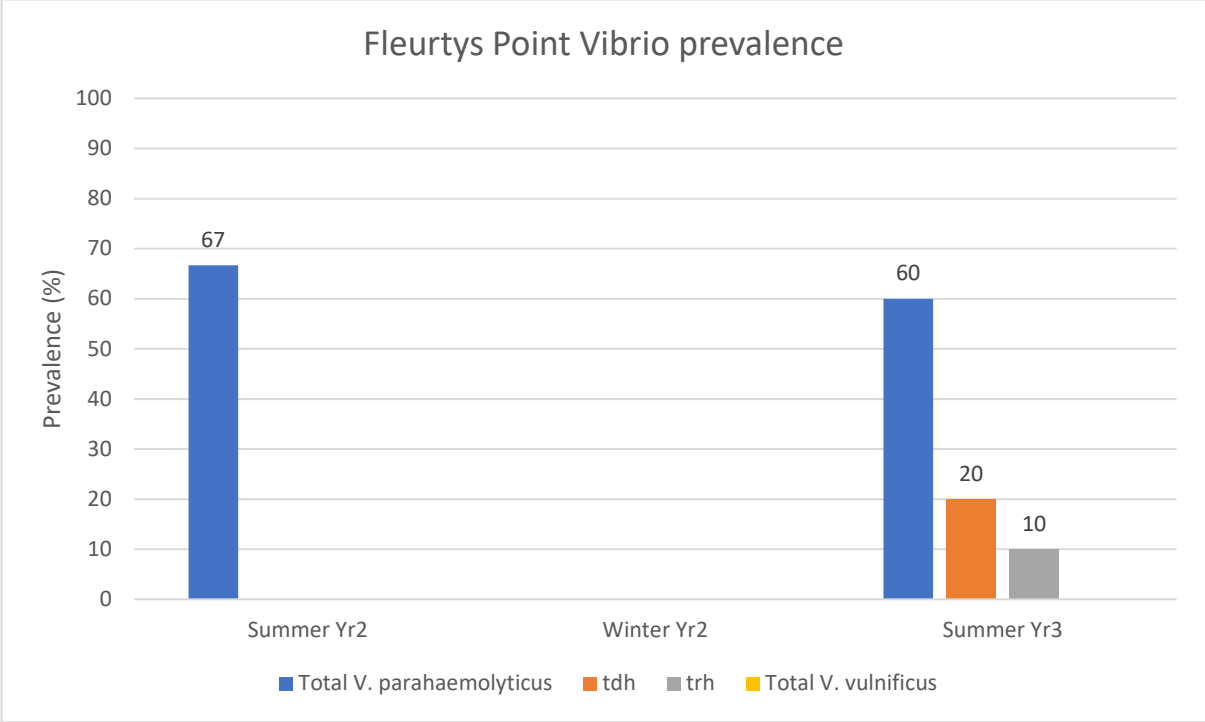

Figure 3: Seasonal *Vibrio* prevalence during the survey period.

No *V. parahaemolyticus* was detected when sampling water was <17°C or when the average and the minimum water temperatures 3-day prior to sampling were, <15°C and <8°C, respectively. No relationship could be established with *V. parahaemolyticus* and sampling of shellfish either above or below the water. Noting that most oysters from Fleurtys Point were collected (n=18/21) below the water, n=8 of these were positive for *V. parahaemolyticus*, while n=1/3 collected above the water were also positive for *V. parahaemolyticus.* No clear relationship between tidal stage and *V. parahaemolyticus* levels could be deduced (Figure 4).

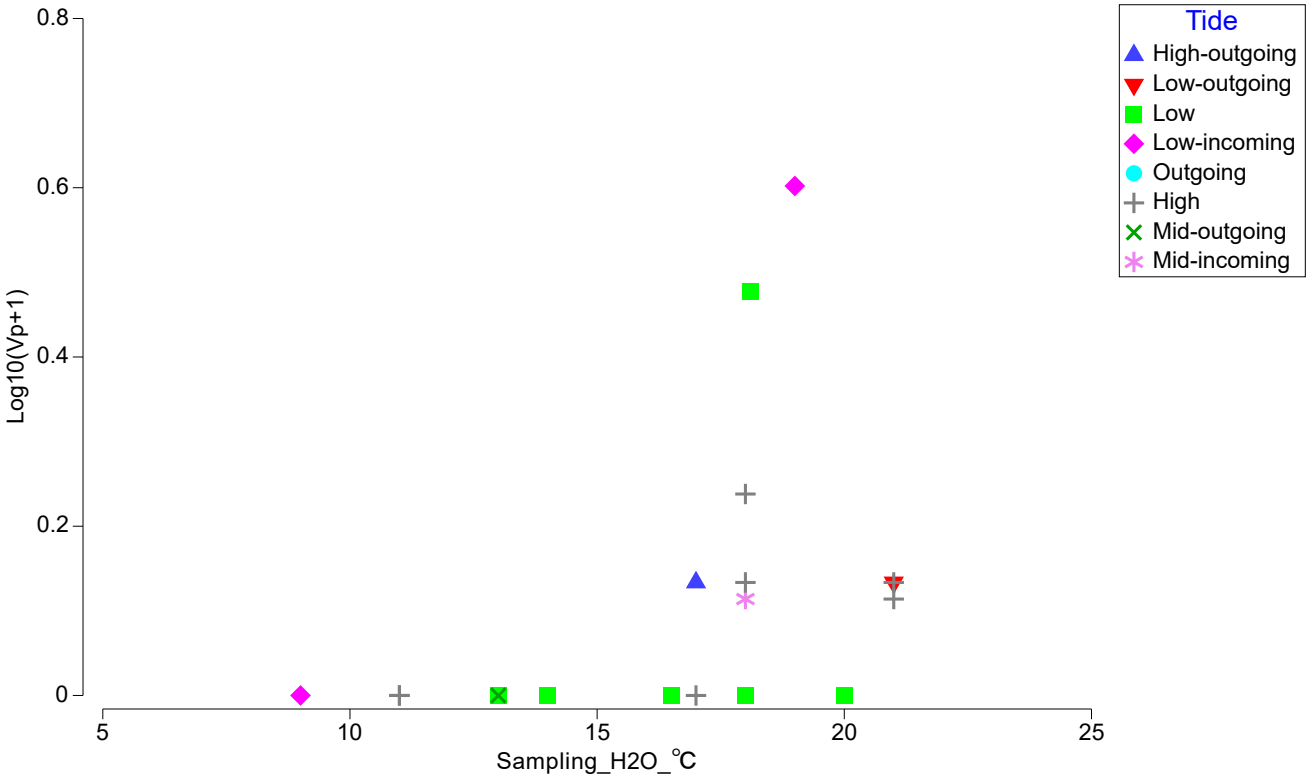

Figure 4: Scatter plot of *V. parahaemolyticus* versus sampling temperature identified by tidal stage of collection.

Environmental and microbiological summary data

Table 1 summarises all the environmental data, while Table 2 summarises all the *Vibrio* data during the survey aggregated into summer/autumn (summer) and winter/spring (winter). A wide range of environmental conditions were recorded in the growing area over the period of the survey. Although *V. parahaemolyticus* was detected in 60% of all summer/autumn samples during the survey, the bacterial levels where low, with a maximum detection of 3 MPN/g detected.

Table 1: Summary table of environmental parameters over the summer/autumn and winter/spring survey periods

	Summer (n=15)					**Winter (n=6)**				
	Min	**Max**	**Average**	**SD**	**n=**	**Min**	**Max**	**Average**	**SD**	**n=**
Temperature (°C)										
Sampling H_2O temp	13.0	21.0	18.0	2.3	15	9.0	14.0	12.0	2.0	6
Sampling air temp	9.0	26.0	19.0	4.8	12	9.5	15.0	13.3	2.3	6
3-day min H_2O temp	8.5	18.5	14.3	3.2	13	8.0	10.5	9.4	1.0	6
3-day max H_2O temp	14.5	23.0	20.0	2.8	13	11.0	14.5	12.3	1.4	6
3-day Av H_2O temp	13.4	19.7	17.1	2.0	13	10.3	13.1	11.5	1.1	6
1-day max air temp	10.7	31.3	20.5	4.3	15	12.3	17.8	14.5	2.1	6
Salinity (PSU)										
Sampling H_2O	32.8	35.3	34.2	0.7	15	30.0	34.1	33.1	1.6	6
Rainfall (mm)										
1-day	0	10.2	1.4	3.0	15	0.4	15.6	5.5	6.7	6
2-days	0	10.2	1.7	3.0	15	0.4	21.6	8.2	8.9	6
3-days	0	58.6	7.4	15.7	15	0.4	21.6	9.3	8.2	6
7-days	0	60.4	14.5	19.8	15	1.6	22.6	11.0	7.6	6
River flow (ML/d)										
Huon River	460	12381	2635.3	3760.1	15	1858.4	56088	16828	21012	6

Table 2: Summary table of *Vibrio* over the summer/autumn and winter/spring survey periods

***Vibrio* enumeration (MPN/g)**						
Vibrio	**Summer (n=15)**			**Winter (n=6)**		
	Min	**Max**	**Positive (n=)**	**Min**	**Max**	**Positive (n=)**
Total *V. parahaemolyticus*	0	3	9	0	0	0
tdh	0	0.36	2	0	0	0
trh	0	0.36	1	0	0	0
Total *V. vulnificus*	0	0	0	0	0	0

Statistical analysis

Temperature

The temperature predictor variables were all positively correlated with one another (Figure 5). The strongest correlations were observed between the average and maximum water temperature 3-days before sampling (R=0.98), followed by sampling and maximum 3-day water temperature (R=0.96), and sampling and 3-day average water temperature (R=0.94). *V. parahaemolyticus* numbers were all positively correlated with the temperature variables, with the highest correlation observed with the minimum water temperature 3-days preceding sampling (R=0.67).

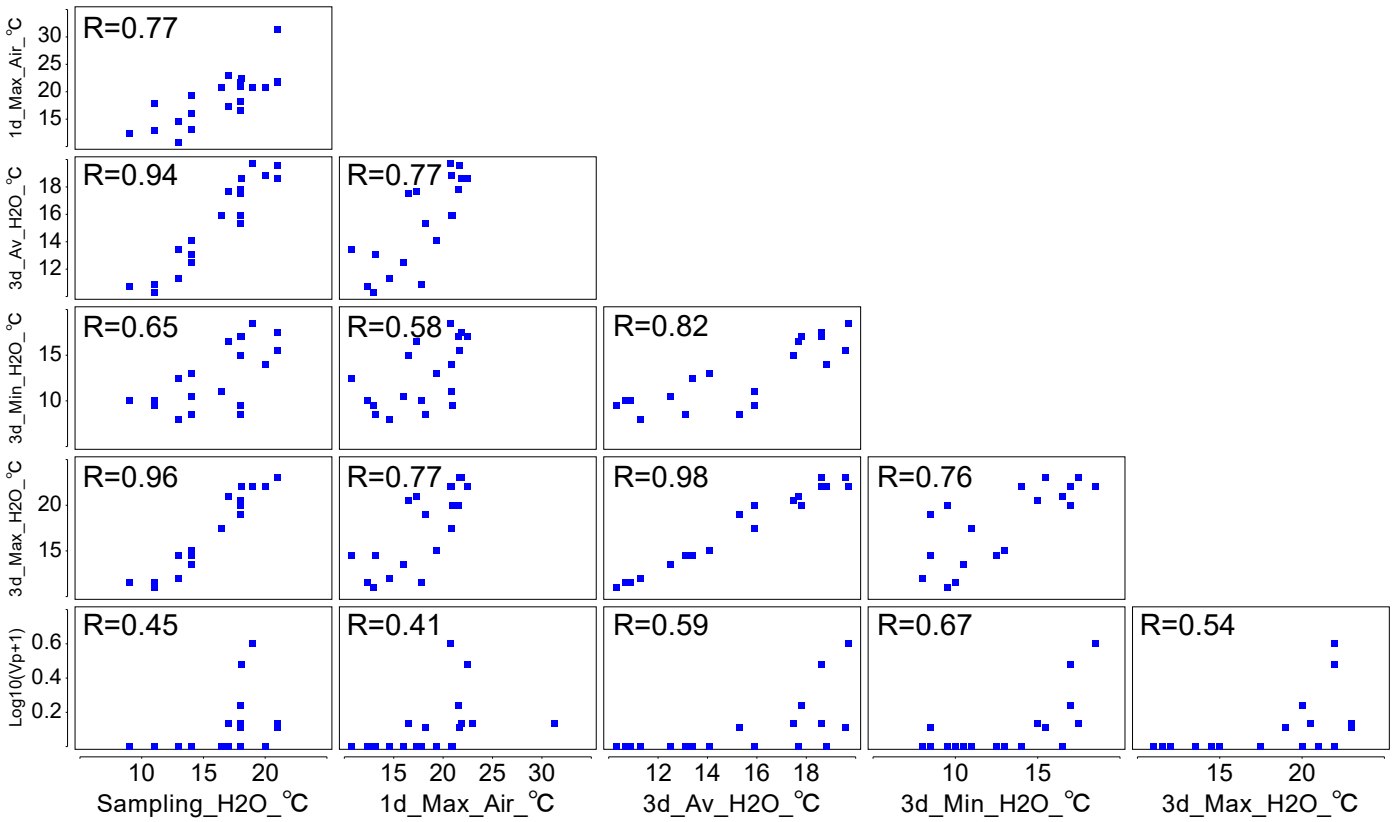

Figure 5: Draftsman's plots of temperature variables and *V. parahaemolyticus*.

Rainfall

Rainfall from 1- to 7-days pre-harvest were all positively correlated to various degrees (Figure 6). The highest inter-correlations were observed between 1- and 2-days rainfall (R=0.94), followed by 3- and 7-days rainfall (R=0.86). *V. parahaemolyticus* levels were weakly negatively correlated with the rainfall predictors. However, the strongest correlations were observed with 7- and 3-day rainfall, respectively.

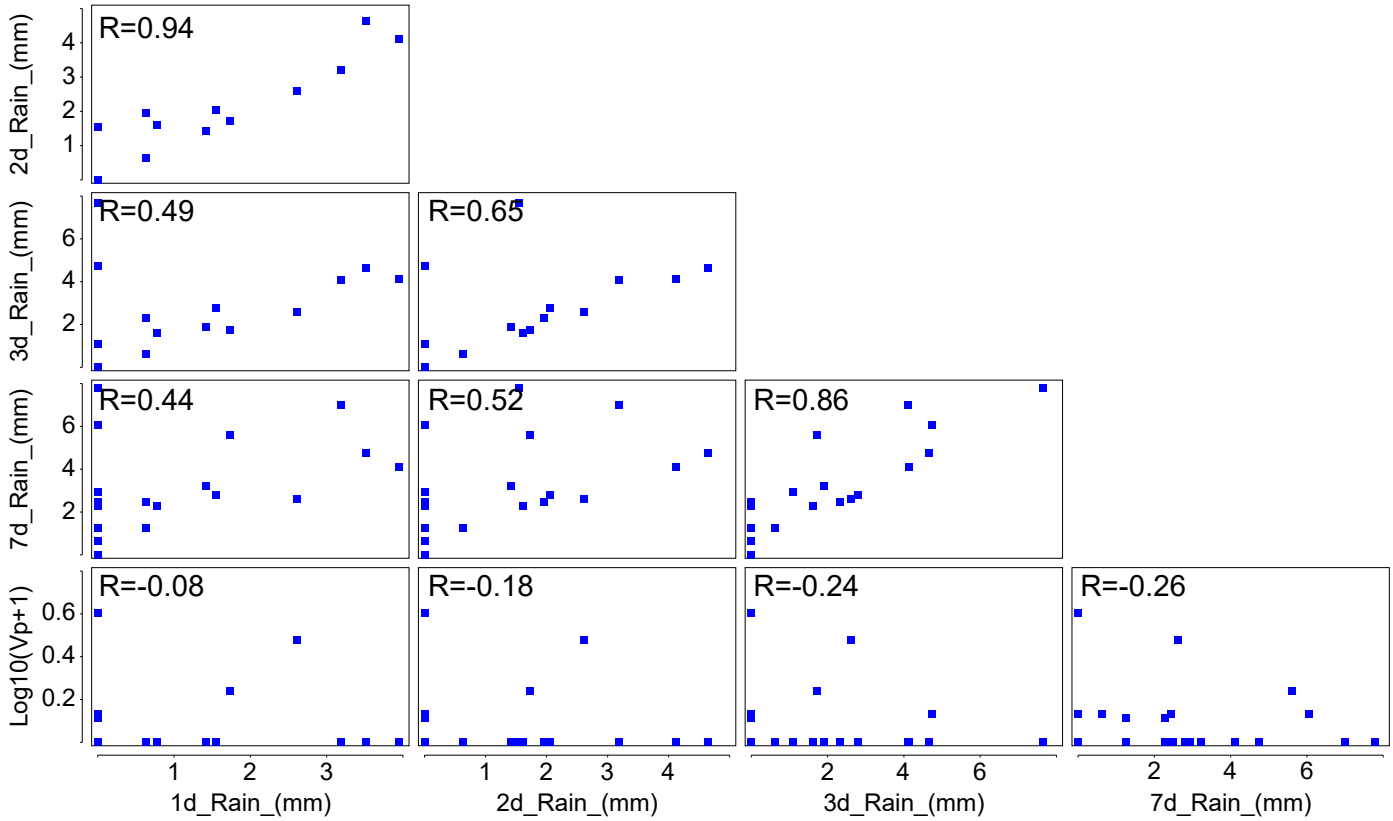

Figure 6: Draftsman's plots of rainfall variables and *V. parahaemolyticus*. Rainfall data was square root transformed.

Salinity and river flow

Harvest water salinity and maximum river flow of the Huon River were weakly negatively correlated (Figure 7). *V. parahaemolyticus* numbers were somewhat positively correlated with harvest water salinity (R=0.31) and negatively correlated with maximum river flow (R=-0.48).

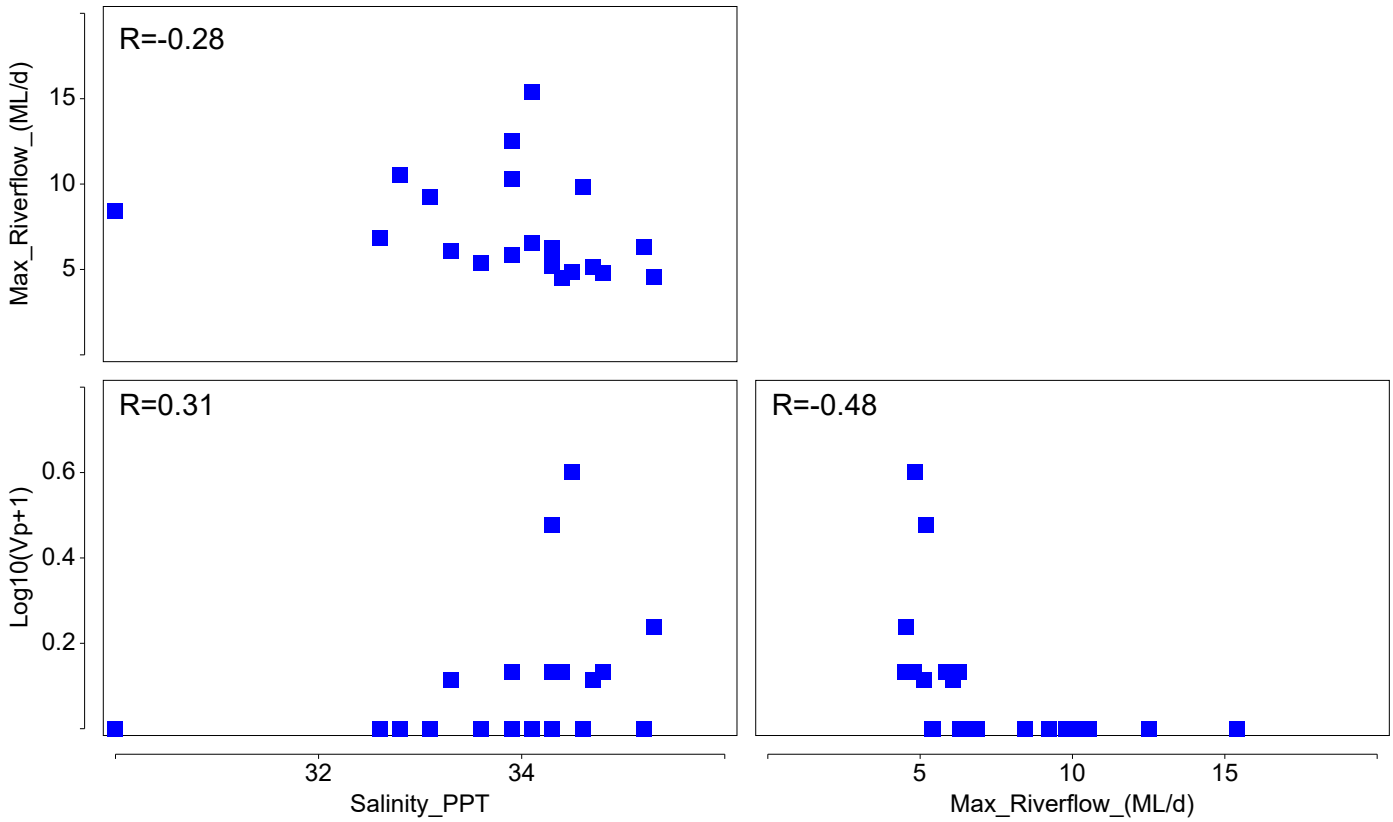

Figure 7: Draftsman's plots of salinity, river flow and *V. parahaemolyticus* variables. River flow data was fourth root transformed.

Linear predictive model generation

The marginal statistical tests in DISTLM showed significant relationships of all temperature predictor variables with *V. parahaemolyticus* numbers. Sampling water temperature (Pseudo-F=4.6, P=0.045), average (Pseudo-F=9.2, P=0.003), minimum (Pseudo-F=13.6, P<0.001) and maximum (Pseudo-F=6.9, P=0.007) water temperature 3-days prior to sampling, as well as, the maximum daily air temperature (Pseudo-F=5.3.1, P=0.031) were all found to be significant drivers individually, explaining 21%, 35%, 44%, 29% and 24% of the observed variability, respectively. All these temperature variables were noted to be strongly correlated (Figure 5). The maximum river flow also showed a significant individual relationship with *V. parahaemolyticus* numbers (Pseudo-F=5.0, P=0.034), explaining 23% of the variability observed. In the most accurate model selection minimum water temperature 3-days prior to sampling alone described 100% of the explained variability in the fitted model, 44% of the total variability in the data cloud (AICc=-74.227 R^2=0.44) (Figure 8).

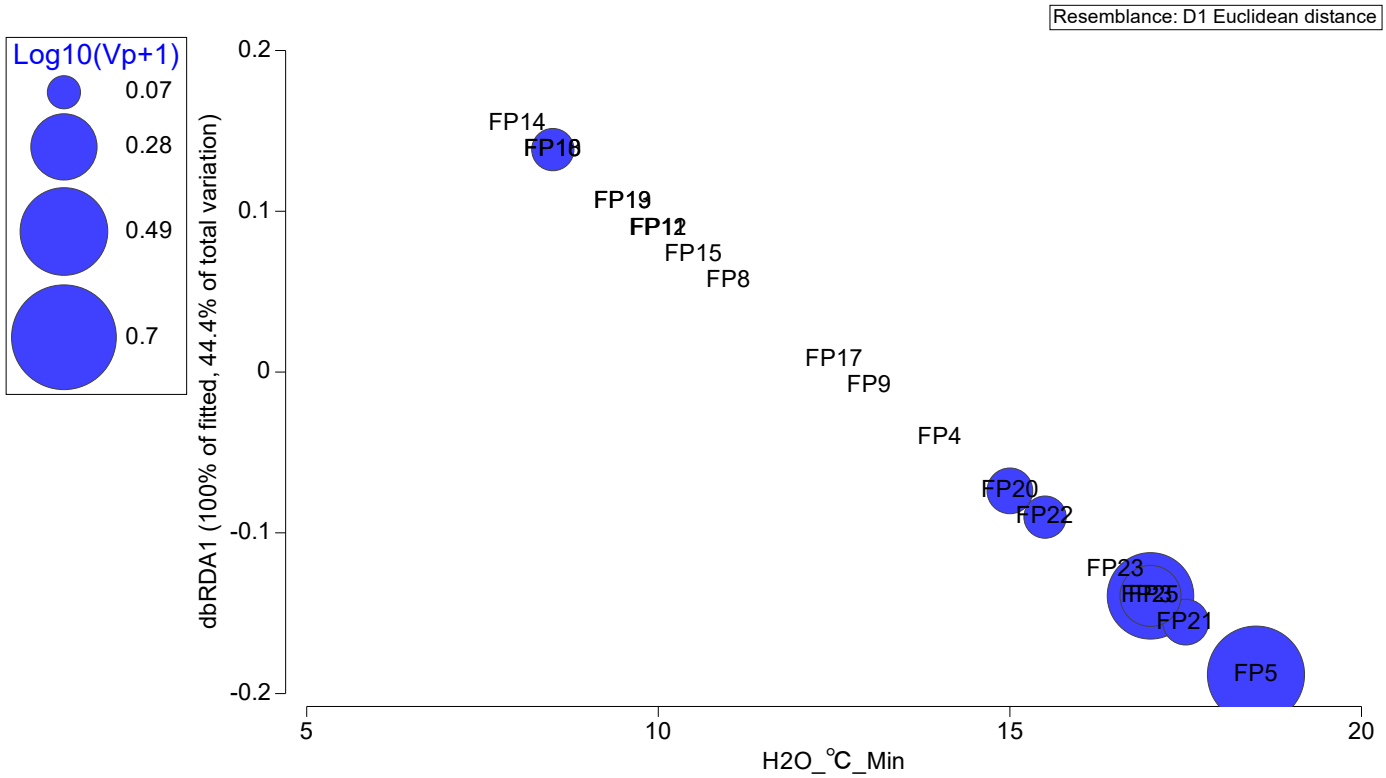

Figure 8: Distance based redundancy analysis (dbRDA) to visualise the DISTLM results of the developed model with the predictor variable minimum water temperature 3-days prior to harvest. Bubbles on plot indicate level of *V. parahaemolyticus* (Vp) detected in shellfish sampled from Fleurtys Point.

The predictive model developed for *V. parahaemolyticus* in Fleurtys Point for oysters was based on the minimum water temperature 3-days prior to sampling:

$$y = -0.3260 + 0.0327x_0$$

Where:

y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = minimum water temperature 3-days prior to sampling (°C)
R^2 = 0.44

Conclusions

- *V. parahaemolyticus* was only detected in Fleutrys Point in in the summer/autumn sampling seasons between 2020/21 and 2021/22 with a prevalence of 60-67%.
- No *Vibrio parahaemolyticus* was detected in the single winter/spring sampling period in 2021.
- Levels of *V. parahaemolyticus* detected during the survey period were very low; ≤3 MPN/g of oysters
- *V. parahaemolyticus* carrying the *tdh* and *trh* genes, often associated with clinical strains, were detected in the summer/autumn season of 2021/2022 with a prevalence of 20% for *tdh* and 10% for *trh*.
- No *V. vulnificus* was detected during the survey period.
- Temperature and maximum river flow were all individually significant indicators of *V. parahaemolyticus* risk in oysters from Fleurtys Point. No *V. parahaemolyticus* was detected when sampling water was <17°C or when the average and the minimum water temperatures 3-day prior to sampling were, <15°C and <8°C, respectively.
- The best predictive model developed for *V. parahaemolyticus* risk in Fleurtys Point was based on minimum water temperature 3-days prior to sampling.

$$y = -0.3260 + 0.0327x_0$$

Where:
y = $\log_{10}$ (*V. parahaemolyticus* numbers + 1) (MPN/g)
x_0 = minimum water temperature 3-days prior to sampling (°C)
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